AN INVESTIGATION OF KOI CARP (*CYPRINUS CARPIO*) MOVEMENT IN THE WAIKATO REGION USING LASER ABLATION OTOLITH MICROCHEMISTRY

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Abstract

The koi carp *Cyprinus carpio* is an invasive fish that has reached high numbers and biomass in the North Island of New Zealand, particularly in the Waikato region. Recent research in Australia has shown that floodplains provide spawning habitat and that recruits are exported from these areas. Recruitment sources and dispersal of New Zealand koi carp have not yet been quantified. This study examined the feasibility of using laser ablation otolith microchemistry to identify koi carp spawning areas and track dispersal of fish.

Water samples from six locations (Lake Waahi, Lake Whangape, Lake Waikare, the Whangamarino River, and the Waikato River at Aka Aka and Rangiriri) were analysed using inductively coupled plasma mass spectrometry (ICP-MS). Significant differences were found in the water concentrations of many elements between sites, indicating possible differences in otolith chemistry of resident fish from these locations. Koi carp were collected from the above locations, as well as from Opuatia Stream, Pungarehu Stream, the Maramarua River and Lake Hakanoa. Concentrations of trace elements in the asteriscus otoliths were analysed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Mean concentrations of at least two elements were significantly different between sites in the edges and nuclei of all koi carp.

A discriminant function analysis (DFA) was carried out using the otolith edge elemental signatures of koi carp. The model used Ba, Sr and Rb concentrations to differentiate between four capture locations: the Waikato River, Opuatia Stream, Lake Waahi, and Lake Waikare and Pungarehu Stream combined. The DFA was able to correctly predict the capture location of 75% of koi carp using otolith edge elemental signatures.

The classification functions created using koi carp otolith edge signatures were then used as a training set to classify otolith nucleus signatures. The otolith

nuclei of 100% of YOY koi carp were classified to their site of capture, suggesting they had not yet dispersed. However, 45% of adult carp had otolith nucleus signatures matching their site of capture. Sixteen of the 20 (80%) adult koi carp caught at Lake Waikare and Pungarehu Stream had nucleus signatures matching their capture sites, indicating that these fish had either originated from this location or returned there after dispersal. Similarly, 55% of otolith nuclei of carp caught from the Waikato River at Aka Aka were classified to the Waikato River, indicating that the fish were of local origin.

Most koi carp caught at Lake Waahi and the Waikato River at Rangiriri had otolith nucleus signatures that did not match their site of capture. Carp caught at Lake Waahi originated from a range of locations in the lower Waikato area. Thirteen of 16 (81%) fish from the Waikato River at Rangiriri had otolith nuclei that were classified to Pungarehu Stream and Lake Waikare.

While carp caught from some areas (Lake Waikare, the Waikato River at Aka Aka) likely originated there, carp caught from other areas (Opuatia Stream, Lake Waahi, the Waikato River at Rangiriri) appear to be of mixed origin. The Waikato River provides koi carp recruits for the Waikato River at Aka Aka, and Lake Waikare and Pungarehu Stream provide koi carp recruits for both the Waikato River and the local area.

Preface and Acknowledgements

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Chapter 1. Introduction

1.1 The common carp, *Cyprinus carpio.*

1.1.1 General introduction

The common carp, *Cyprinus carpio* (Family Cyprinidae), originates from Eastern Europe and has spread through much of the world, including New Zealand (Balon, 1995; McDowall, 2000; Koehn, 2004). *C. carpio* is considered to be the fish species of most concern in New Zealand (Chadderton et al., 2003). The strain present in New Zealand is the koi carp (Figure 1.1), a highly coloured strain originating from domestic ornamental stocks (McDowall, 2000). The koi carp is likely to have been brought into New Zealand in the 1960s along with imports of other fish, and released either accidentally or deliberately into the wild (McDowall, 1997 & 2000). This species is farmed for food and valued as an ornamental fish in many countries, though its main value in New Zealand is as a sport fish for coarse anglers and bow hunters.

Figure 1.1 The koi carp *Cyprinus carpio*, caught in Lake Whangape.

The koi carp is now distributed widely throughout the North Island, with many recorded populations in the Waikato region in the upper central North Island (Figure 1.2). A population of koi carp was detected in a farm pond in Nelson in 2000, but this has since been eradicated (Chadderton et al., 2003). In New Zealand, koi carp inhabit the slow-flowing parts of rivers and streams, weedy backwaters, willow fringes and shallow areas of lakes (McDowall, 2000; Osborne, 2006). In the Waikato region, the koi carp can comprise up to 90% of fish biomass at individual locations (Osborne, 2006). Biomass estimates range from 1,200 kg ha^{-1} in the Waikato River to over 2,000 kg ha^{-1} in the outlet of Lake Kimihia (Osborne, 2006).

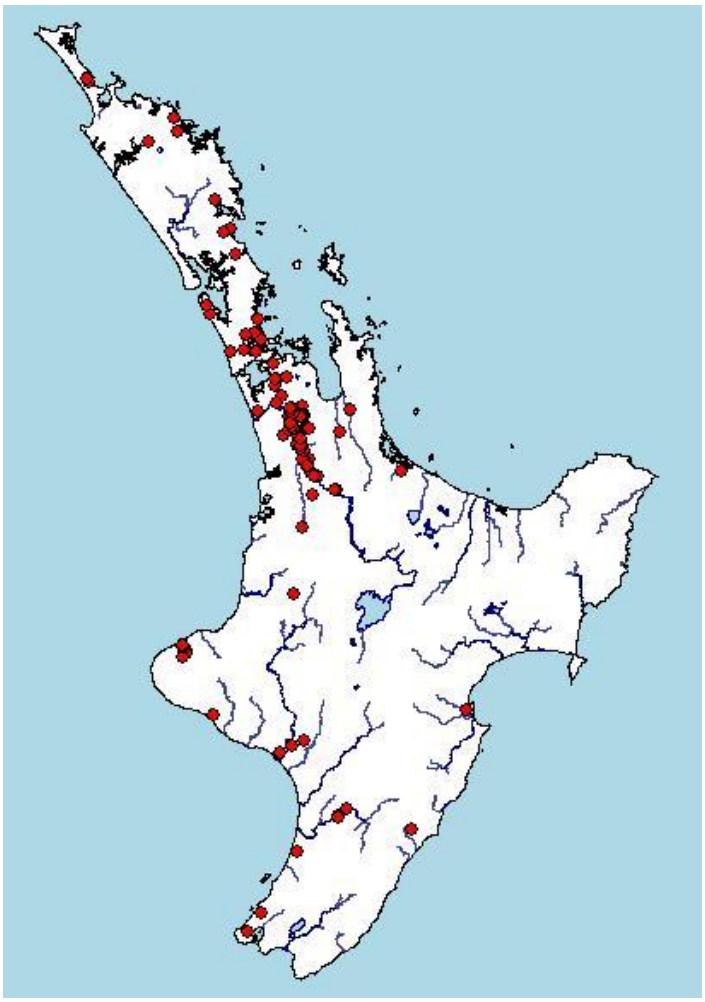

Figure 1.2 Recorded occurrences of koi carp in the North Island, New Zealand. Source: New Zealand Freshwater Fish Database (NIWA; Jowett, 2007)

The koi carp has long been recognised as a threat to ecosystems in New Zealand (Hanchet, 1990) but little has been done to limit its spread. It is classified as an unwanted organism under the Biosecurity Act (1993), and a containment zone for this species exists in the upper North Island (Dean, 2003). Within this zone it is legal to fish for koi carp, but if caught, they must be killed immediately. It is illegal to hold live koi carp without a permit from the Department of Conservation.

The koi carp possesses many attributes common to invasive species, such as a broad diet, wide environmental tolerances, high fecundity, short generation times, and rapid growth (Koehn, 2004). It is tolerant of a broad range of environmental disturbances including pollution, anoxia and turbidity (McDowall, 2000). These attributes are likely to give koi carp the ability to outcompete native fish (Koehn, 2004). In the Waikato region of New Zealand, koi carp are highly fecund; females produce an average of 299,000 oocytes (Tempero et al., 2006). They are capable of multiple spawnings during their lifetimes, and females are able to spawn more than once during a single spawning season (Tempero et al., 2006). In the Waikato, males usually reach a maximum age of 8 years, and females 12 years (Tempero et al., 2006).

The koi carp's habitat requirements for reproductive success are not well known in New Zealand. In Australia, floodplain habitat is thought to be the source of most carp recruits, suggesting that flooding events are important for carp reproduction (King et al., 2003; Crook & Gillanders, 2006; Stuart & Jones, 2006a). Carp in Australia show higher recruitment success in warm, non-regulated lowland environments, where flood events are more frequent (Driver et al., 2005). Larval carp abundance has been reported to increase after flooding events (Stuart and Jones 2006a) and downstream from floodplain habitat (King et al., 2003).

1.1.2 Impacts of carp

Carp have negative effects on aquatic ecosystems that are most often attributed to their feeding method. Carp are benthivorous as adults and feed by ingesting sediment, filtering out food particles, and expelling the rest (Sibbing et al., 1986). Benthivorous fish affect biotic and abiotic aspects of ecosystem function, including nutrient recycling, water clarity, macrophytes, phytoplankton, zooplankton and macroinvertebrate assemblages (Northcote, 1988).

The most commonly reported effect of carp is an increase in water turbidity caused by an increase with carp biomass (Roberts et al., 1995; King et al., 1997; Loughleed et al., 1998; Zambrano & Hinojosa, 1999; Driver et al., 2005). Increased turbidity and nutrient levels can cause a shift in primary production in lakes from macrophyte-dominated to phytoplankton-dominated (Scheffer, 1993). An increase in turbidity could also reduce radiation available for photosynthesis or impair visually orientated predation by other species (Koehn, 2004).

Carp increase suspended sediment in the water column, increasing turbidity, light penetration, conductivity, and phosphorous concentrations (Driver et al., 2005). Zambrano and Hinojosa (1999) reported that enclosures containing over 0.8 individuals m^{-2} reached a turbid state more quickly than those containing less than 0.8 individuals m^{-2}. These authors attributed the increase in turbidity to the carp's direct disturbance of the sediment and interruption of the sediment consolidation process. Another study also attributed an increase in water column total phosphorous and suspended solids to increased sediment resuspension by carp (Parkos et al., 2003). However, Lamarra (1975) found that the digestive activities of carp, not the physical disturbance of the sediment, caused an increase in water column total phosphorous and chlorophyll *a* in lake enclosures. This apparent contradiction was explained by Driver et al. (2005), who found the effects of carp can be driven by size, rather than biomass. Smaller carp increase phosphorous by excretion, while larger carp increase phosphorous by sediment disturbance (Driver et al., 2005). These authors also found that if all size classes were represented, nitrogen regeneration increases with carp biomass. An

increase in total phosphorous with increased carp biomass was also reported by King et al. (1997). Loughleed et al. (1998) reported an increase in turbidity, total phosphorous and total ammonia with increased carp biomass. King et al. (1997) found higher phytoplankton biomass in billabong areas stocked with higher numbers of carp, which would also contribute to high turbidity. In New Zealand, no studies have been undertaken to examine the effect of koi carp on lake and stream water quality. However, in small North Island lakes in New Zealand, the presence of exotic fish has been correlated with turbidity (Rowe, 2007).

Invertebrate populations can also be affected by carp. Parkos et al. (2003) reported a reduction of zooplankton and macroinvertebrate assemblages in the presence of carp. Loughleed et al. (1998) also found that increased carp biomass was associated with decreased zooplankton numbers.

In shallow lakes such as those in the Waikato region, macrophytes play an important role in buffering the effects of eutrophication (de Winton et al., 2003). Carp can negatively affect macrophyte growth by disturbing roots during feeding, damaging plants with soft or shallow roots more severely (Fletcher et al., 1985; Roberts et al., 1995; Zambrano & Hinojosa, 1999). It is also possible that carp reduce light available for macrophyte growth (Parkos et al., 2003), though this should not pose a problem in very shallow water (Zambrano & Hinijosa, 1999). Williams et al. (2002) attributed macrophyte loss not to physical disturbance, but to a decrease in light caused by increased epiphyton growth. Growth of nitrogen limited epiphyton was likely to have been stimulated by carp nitrogen excretion (Williams et al., 2002).

1.1.3 Carp movement

Migration between feeding, spawning, and refuge habitats is common in fish (Wells et al., 2003), and identifying these migrations is a principal concern of fisheries research (Elsdon & Gillanders, 2004). Though carp usually remain within home ranges, they capable of wide dispersal. Stuart and Jones (2006b) reported that a large proportion of tagged adult carp (80%) moved less than 5 km during a

mark-recapture study in the Murray-Darling Basin, Australia. Some individuals showed a much larger scale of movement; over 7% moved 100 km or more (Stuart and Jones, 2006b). Juvenile carp moved shorter distances than adult carp. Of the six juvenile carp tracked by Stuart and Jones (2006b), four moved under 1 km, one moved between 1.1 and 1.5 km upstream, and one moved between 5.1 and 50 km downstream. This shows that the distances travelled by juvenile carp should be considered when examining carp movement.

Similar to Australian carp, koi carp in New Zealand show a high degree of site fidelity (Osborne et al., in press). In a recent mark-recapture study in the Waikato River, 86% of fish were recaptured within 5 km of their release site (Osborne et al., in press). Though the average distance travelled was 4 km, the greatest distance travelled was 75 km during 913 days at liberty (Osborne et al., in press). The length of the fish did not affect distance travelled, but fish at liberty longer than 24 months travelled significantly further than those at liberty less than 24 months (Osborne et al., in press). Only fish with fork lengths greater than 180 mm were tagged, so no information was gained on the movement of sub-adult and juvenile carp (Osborne et al., in press). However, it is possible that movement was underestimated in this study. Preliminary results from a radio tagging study of koi carp in the lower Waikato region showed that of 12 fish tagged, 10 migrated an average of 44 km (males) and 30 km (females) during 148- 179 days at liberty (Daniel, A., personal communication, 18 June 2008).

Larval fish drift can play an important part in new habitat colonisation as they may be distributed over wide areas by currents (Humphries & King, 2003). However, in Australia, carp usually spawn in still-water environments such as floodplain wetlands, isolated anabranches and billabongs (King et al., 2003) where drift is unlikely. Humphries and King (2003) classify carp as facultative drifters; although larval drift is not essential for carp survival, it may play an important part in dispersal and colonisation of new habitats. Meredith et al. (1992) reported drift of larval goldfish and koi carp down the Waikato River in the spring. Carp larvae were thought to drift from upstream spawning and nursery sites (Meredith et al., 1992). Boubée et al. (2004) estimated that 38,000

cyprinids (approximately 85% koi carp and 15% goldfish) moved from Pungarehu Canal into Lake Waikare via the Waikare Fish Pass between November 2003 and March 2004. Further, they noticed that several fish moved in and out of the lake on multiple occasions.

Larval and juvenile fish often occupy different habitats to adult fish. Movement between natal and adult habitats can be quantified using tagging studies, but because larval fish may have a low survival rate, many thousands of fish must be tagged. The cost of such operations can be prohibitive, and return rates of marked fish can be very low (Elsdon & Gillanders, 2004). Larval dispersal may also primarily occur during rare events, such as floods, which may not be included in the duration of a tagging study (Wells et al., 2003). In the present study, young carp were found to be difficult to capture and impossible to find in high numbers; this has also been noted by previous researchers (Tempero et al. 2006). Otolith microchemistry provides a chronological record of the fishes' environment, providing information about movement with less difficulty than tagging. This allows the study of movement over the entire life span of the fish without the need to mark and recapture large numbers of fish.

1.2 The goldfish, *Carassius auratus.*

Goldfish, *Carassius auratus*, were released into the wild in New Zealand "in very early colonial times" (McDowall, 2000, p. 139). Goldfish and koi carp occupy similar habitats, but goldfish are distributed more widely; they are found in some areas of the South Island as well as being widely distributed throughout the North Island (McDowall, 2000). McDowall (2000) states that goldfish are unlikely to have any negative effects on the environment, but the regional council, Environment Waikato, has recently added this species to its 2007-2012 Regional Pest Fish Management Strategy (Environment Waikato, 2007). This means that they will be subject to the same monitoring and control as koi carp (Environment Waikato, 2007). Goldfish can reach extremely high densities (15,000-17,000 fish ha^{-1}), and can increase turbidity and cause extensive damage to aquatic

macrophytes by grazing (Richardson et al., 1995). In addition, the growth of nuisance cyanobacteria such as *Microcystis aeruginosa* can be stimulated by passing through goldfish guts (Kolmakov & Gladyshev, 2003). Goldfish were recently removed from the Vasse River in Australia because of their high growth rates and effects on native wildlife (Morgan et al., 2005). Goldfish were included in the present study for comparison with carp and to assess the feasibility of using otolith chemistry methods to study this species.

1.3 Otolith biology

Most vertebrates possess paired calcium carbonate structures in the inner ear which are used for balance and hearing, aiding stimulation of hair cells in the inner ear (Popper et al., 2005). In teleost fish they are called otoliths or ear stones. Otoliths are comprised almost completely of $CaCO_3$, with other elements present in trace amounts. The elements Na, Sr, K, S, N, Cl and P are present at levels below 100 ppm, whereas most other trace elements are present at levels less than about 10 ppm (Campana, 1999). New material is added to otoliths throughout the fishes' life, creating a series of rings which can be used to age the fish. Otolith research has previously focussed on ageing, but current efforts are also concerned with using otoliths in microstructural and microchemical applications (Campana, 2005).

The otolith examined in this study was the asteriscus, which is the largest otolith in ostariophysarian fishes such as goldfish and carp (Secor et al., 1991). The asteriscus is located in the lagena of the inner ear and is composed of the mineral vaterite, not aragonite, which makes up the sagittus and lapillus otoliths (Secor et al,. 1991). Unlike other bony structures such as spines and scales, otoliths are continuously deposited and metabolically inert, and therefore not reabsorbed during periods of stress (Campana, 1999). New material is deposited continuously on the otolith surface, even when somatic growth has stopped (Maillet & Checkley, 1990).

1.4 Otolith microchemistry

Otolith element concentrations give a record of the environmental and physiological conditions the animal has experienced during its lifetime. Elements from the surrounding water are deposited to greater or lesser degrees in the matrix of the otolith during precipitation from the endolymph (Campana, 1999). Therefore, providing groups of fish have been subjected to different environmental conditions, groups should be distinguishable on the basis of otolith trace elemental concentrations. The combination of elements in the water or otolith is referred to as the chemical or elemental signature. Because otolith material is laid down continuously, providing a chronological record of the fishes' life, elemental concentrations can be analysed from the nucleus to the edge of the otolith, allowing "reconstruction of migration pathways structured by age" (Campana, 1999, p. 285).

Elements are not deposited equally into the otolith matrix. Ba and Sr will substitute Ca in the $CaCO_3$ lattice because they are also divalent metals (Speer, 1983). Sr is the most commonly studied element in fish otoliths, and can serve as a record of both physiological and spatial changes (Clarke & Friedland, 2004). Otoliths are surrounded by endolymphatic fluid, which is metabolically regulated and isolated from the outside environment (Campana, 1999). The concentrations of most elements found in the otolith are not always directly related to water concentrations due to metabolic regulation and other factors (Kalish, 1989; Campana & Thorrold, 2001). Water temperature and salinity can also influence otolith element concentrations (Bath et al., 2000; Elsdon & Gillanders, 2004). However, water chemistry seems to be the predominant determinant of otolith chemistry. Ratios of Sr/Ca and Ba/Ca found in fish otoliths are proportional to the ratios of these elements in the environment (Farrell & Campana, 1996; Bath et al., 2000; Crook et al., 2006). Li, Mn, Ba and Sr are all influenced by water concentration and temperature, whereas Mg seems to be highly metabolically regulated (Campana et al., 2000). However, it is not necessary to link otolith element concentrations back to water chemistry, since the aim is to learn about

fish migration, and not to use otoliths to infer water chemistry (Campana & Thorrold, 2001).

Ontogeny can also affect element concentrations; elevated levels of Mn were found in the nuclei of clupeid otoliths compared to the otolith edges (Brophy et al., 2004). The cause of the elevated Mn levels was unknown, as environmental Mn levels were not high (Brophy et al., 2004). Otolith chemical concentrations differ between embryonic and larval fish (Chittaro et al., 2006) and between larval and young juvenile fish (Fowler et al., 1995) raised in the same environments. However, somatic growth rate did not affect salmon otolith microchemistry (Clarke & Friedland, 2004). Elsdon and Gillanders (2005) found no influence of fish age on otolith concentrations of Ba and Sr in adult black bream *Acanthopagrus butcheri*.

Applications of otolith microchemistry include tracking migrations between fresh and salt water (Secor, 1992; Arai & Hirata, 2006), and identifying natal areas in estuarine (Miller, 2007) and freshwater environments (Wells et al., 2003; Brazner et al., 2004; Crook & Gillanders, 2006; Clarke et al., 2007). Otolith microchemistry can be used to identify natal areas of adult fish populations. First, otolith edge regions of fish from a particular location are analysed, identifying a characteristic chemical signature for that area. Otolith nucleus element concentrations are then compared to the edge element concentrations. If nucleus and edge signatures of a particular fish are similar, the fish likely originated from the area where it was caught. If they do not match, the fish likely originated from another area and migrated to its present location. This technique was used by Miller (2007) to identify natal sites of juvenile estuarine fish. In addition to tracking migration in marine environments, this method can be used to determine the importance of putative natal areas in freshwater. Brazner et al. (2004) found that yellow perch originating from bays and coastal wetlands of Lake Superior, USA could be correctly assigned to their home location using otolith microchemistry. Crook and Gillanders (2006) found that the otolith nucleus signatures of fish in the Murray River matched the otolith edge

signatures of fish caught in nearby floodplain lakes, indicating that river fish had originated from the lakes.

1.5 Laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS)

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) can be used to measure the abundances of a wide range of elements in solid samples with fine spatial resolution, and good accuracy and precision (Jackson et al., 1992). Minimal sample preparation is required, and many samples can be processed in a short amount of time. LA-ICP-MS gives results showing a close linear relationship to results from electron microprobe and X-ray analysis (Arai & Hirata, 2006). Samples are typically placed inside a closed chamber, and the laser is used to vaporise the sample, which is carried using Ar gas to the ICP-MS. This technique has been used in a wide range of applications, including fisheries biology, sediment analysis, plant material analysis, and archaeology (Durrant & Ward, 2005).

1.6 Aims and objectives

Koi carp have reached high numbers in New Zealand, and have spread rapidly through the North Island. Due to the carp's possible negative impacts on New Zealand wildlife, it is vital to limit their spread, and control attempts may be planned for the future. If koi carp numbers are to be controlled, a thorough understanding of their local ecology is needed, particularly the conditions necessary for their reproductive success. Recruitment sources and dispersal from these sources have not yet been identified for New Zealand koi carp.

Likely sources of recruits include the Whangamarino wetland and riverine lakes in the lower Waikato catchment such as Whangape, Waikare, and Waahi. It is possible that these recruits move from natal areas into the Waikato River and may undertake further movement during their lifetimes. The objectives of this

study were to determine whether otolith elemental signatures could be used to identify important spawning locations in the Waikato area, and to characterise dispersal from these locations.

Chapter 2. Methods

2.1 Study area

The Waikato River (Figure 2.1), at 450 km long, is the longest river in New Zealand, and one of the most severely affected by human development (Chapman, 1996). It drains New Zealand's largest lake, Lake Taupo, which has a volume of 59 km^3 (Chapman, 1996). The Waikato River has a catchment area of 11,395 km^2 (Duncan & Woods, 2004), which has been heavily modified by agriculture, forestry, and urban development (Chapman, 1996). Geothermal areas in the upper catchment also affect river water quality and chemistry (Chapman, 1996). Discharges from large lakes in the catchment including Lake Taupo, Lake Waikare and Lake Waahi are regulated, modifying the river's flow regimes. Flow is also modified by eight hydroelectric dams along the river. The mean discharge of the Waikato River is 340 $m^3 s^{-1}$, with the highest flows typically occurring in July and August (Duncan & Woods, 2004). Specific mean annual floods are low (60-70 L s^{-1} km^{-2}), and the frequency of events with greater than 3 times the median flow is 0.4 events $year^{-1}$, due to flow regulation and groundwater storage in pumice (Duncan & Woods, 2004).

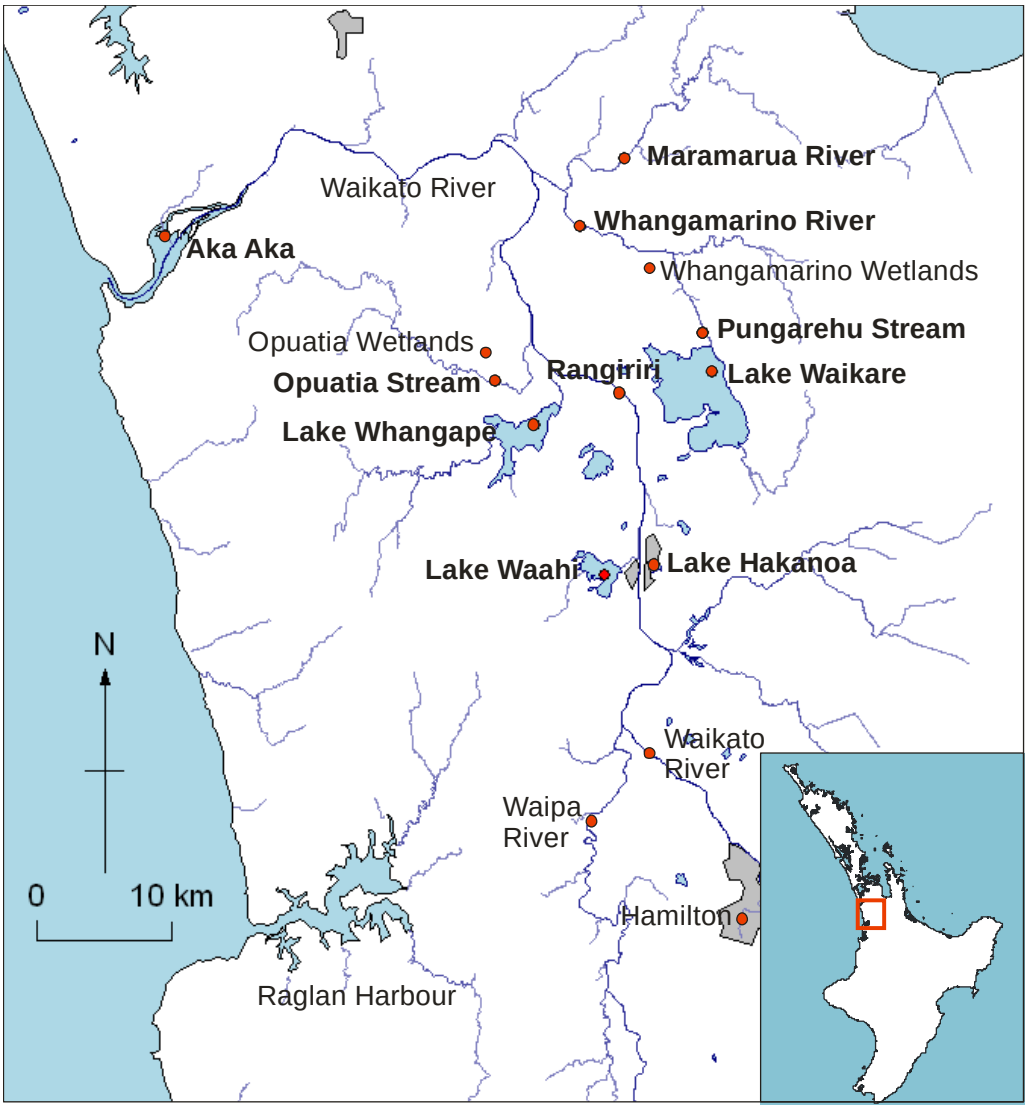

Figure 2.1 Sample site locations in the lower Waikato region, North Island, New Zealand. Fish capture sites are indicated in bold text.

The Whangamarino Wetland (Figure 2.1) has been listed as internationally significant under the Ramsar Convention on Wetlands, and has been described as the most important freshwater habitat in New Zealand (Cromarty & Scott, 1996). One of the first significant populations of koi carp was discovered in the wetland in the 1980s (McDowall, 1997). The Whangamarino Wetland previously covered 10,320 ha (Department of Conservation, 2007) but the Lower Waipa Waikato Flood Protection Scheme in 1961 allowed draining of some land for farming (Cromarty & Scott, 1996), and the wetland now occupies 7,100 ha (Environment Waikato, 2008). The wetland complex contains peat bogs,

swampland, open water and river systems (Cromarty & Scott, 1996). In the mid 1990s, the average number of dry days in the wetland per year was 220, increased from 77 days prior to draining (Reeves, 1994). Since then a rock rubble weir was built in the outlet of the Whangamarino River to reinstate 1960s summer water levels in the wetland (Environment Waikato, 2008).

Whangamarino Wetland is drained by the Maramarua River, the Whangamarino River and Reao Stream (Figure 2.1). The wetland's catchment area is 48,900 ha in non-flood conditions, but it also receives water from Lake Waikare in flood conditions (Cromarty & Scott, 1996). Pungarehu Stream (Figure 2.1), a small man-made channel linking Lake Waikare and Whangamarino Wetland, was created in the 1960s to facilitate flood management. During flood events, Waikato River flood waters are diverted via a spillway at Rangiriri into Lake Waikare. A stop bank and control gates let stored water drain from Lake Waikare into the Whangamarino Wetland and back into the Waikato River after flood waters have receded (Beyá, 2005).

Opuatia Wetland (Figure 2.1), like Whangamarino Wetland, is a peat bog dominated by the wire rush *Empodisma minus* and willows, *Salix* spp. (Campbell & Jackson, 2004). It is drained by Opuatia Stream.

The riverine lakes Lake Hakanoa, Lake Whangape, Lake Waikare and Lake Waahi were sampled in this study (Figure 2.1). Most of the lake catchments in the Waikato region have been heavily modified by draining of surrounding wetlands, clearing of native vegetation, and regulation of water levels (Boswell et al., 1985). Pastoral and residential development is common, and many lakes in the lower Waikato region have been affected by the input of coal mining wastes (Barnes, 2002).

Lake Waikare, with a surface area of 3,442 ha, is the largest lake in the lower Waikato catchment (Boswell et al., 1985). It has a maximum depth of 1.8 m and a mean depth of 1.5 m, and drains to the Whangamarino Wetland via Pungarehu Stream (Boswell et al., 1985). Submerged aquatic plant populations collapsed between 1977 and 1979, leaving the lake in a turbid state, with extremely high

suspended sediment levels (Barnes, 2002). Lake Waikare is hypertrophic, with a trophic level index (TLI) of 6.61 (Barnes, 2002).

Lake Whangape is the second largest lake in the lower Waikato catchment, with a surface area is 1,450 ha, a mean depth of 1.5 m and a maximum depth of 3.5 m (Boswell et al., 1985). Lake Whangape drains to the Waikato River via the Whangape Stream. Lake Whangape's catchment is pastoral, but the lake has also received inputs from mining activities in the past (Barnes, 2002). Macrophyte populations in Lake Whangape collapsed in 1987 (Champion et al., 1993), and the lake is classified as supertrophic, with a TLI of 5.69 (Barnes, 2002).

Lake Waahi has a surface area of 522 ha and a maximum depth of 5 m (Boswell et al., 1985). The lake discharges to Waikato River via a controlled outlet in Waahi Stream. Lake Waahi's catchment is pastoral, but the lake still experiences effects from historical coal mining activities in the area (Barnes, 2002). Lake Waahi is supertrophic, with a TLI of 5.37 (Barnes, 2002). Macrophyte populations collapsed in 1978-79 (Boswell et al., 1985).

Lake Hakanoa is a small lake (52 ha) situated in Huntly township, with a maximum recorded depth of 3.2 m (Boswell et al., 1985).

The fish used in this study were caught from Opuatia Stream, the Maramarua River, the Whangamarino River, the Waikato River at Aka Aka and Rangiriri, Lake Hakanoa, Lake Whangape, Lake Waikare and Lake Waahi (Figure 2.1). Two areas in the main Waikato River were sampled: Aka Aka near the Waikato River mouth at Port Waikato, and Rangiriri, approximately 60 km further upriver (Figure 2.1).

2.2 Fish capture

Fish were caught using a variety of methods. Fifty-three adult koi carp were collected from the 2007 World Koi Carp Classic, a koi carp bow fishing competition held by Lake Waahi near Huntly, on 3 and 4 November, 2007. Sampled fish were caught in the Whangamarino River near Falls Rd (*n*=4), the Waikato River at Rangiriri (*n*=10) and Aka Aka (*n*=11), Lake Hakanoa (*n*=1), Lake

Waahi (*n*=9), Lake Whangape (*n*=9), and Lake Waikare (*n*=9). The exact location of capture was ascertained by interviewing bow fishers.

Fourteen koi carp (119- 620 mm fork length) and 10 goldfish (67- 211 mm F.L.) were caught by boat electric fishing. The electric fishing boat is 4.5 m long and equipped with a 6 kilowatt Honda-powered custom-wound generator and a 5 kilowatt gas-powered pulsator (Smith-Root, Inc., model 5.0 GPP). Pulses of direct current are emitted at 60 pulses per second, and the power output is typically between 2 and 4 amps root mean square, dependent on water conductivity (Hicks et al., 2006). Fishing passes were recorded using a Lowrance GlobalMap® 2400 boat-mounted GPS system.

Nineteen koi carp and four goldfish were caught by backpack electric fishing in the margins of Lake Waikare and in Pungarehu Stream near the Lake Waikare fish pass. Sixteen carp otoliths and their corresponding length and weight data were obtained from a previous study carried out by Tempero et al. (2006). Six adult carp were obtained from Opuatia Stream as by catch from the eel fishery.

All fish were quickly euthanised after capture in an ice slurry. Fish were weighed using a digital balance and the fork length was measured to the nearest millimetre. Fish were sexed where possible by gonad inspection. The heads were then removed from the fish and individually bagged, labelled using waterproof paper, and chilled or frozen until otolith extraction. Adult koi carp were distinguished from goldfish by the presence of barbels (Taylor & Mahon, 1977). Since juvenile koi carp and goldfish can be easily mistaken for each other, fish smaller than 100 mm F.L. were identified by counting lateral line scales on the left side. This is a reliable method for distinguishing the two species as well as hybrids (Taylor & Mahon, 1977). Carp have between 37 and 40 lateral line scales, and goldfish have between 29 and 31 (Taylor & Mahon, 1977).

The length of 140 mm was used to distinguish young fish from adult fish; although carp do not reach maturity until 250-300 mm, carp shorter than 140 mm are likely to be less than one year old (Tempero et al., 2006). The age of

young-of-the-year koi carp was verified by the absence of an annulus on the otolith.

Studies have shown that sample preparation can affect the results of otolith microchemistry analysis. The length of time before otolith extraction and whether the fish or otoliths are stored in alcohol can influence results (Milton & Chenery, 1998; Proctor & Thresher, 1998). All fish in this study were frozen after capture or processed within a few hours, removing the need to preserve in alcohol.

2.3 Otolith sample preparation

Otolith extraction and preparation methods were based on those described by Secor et al. (1991). Asteriscus otoliths were removed using the open-the-hatch method (Secor et al., 1991). This involved making a cranio-caudal cut into the skull using a hacksaw for large fish, or scalpel or knife for smaller fish. The otoliths were then removed using forceps. Although the lapillus or sagittus otoliths are more commonly used for microchemical analysis, the asteriscus is the largest otolith in ostariophysarian fishes such as carp (Secor et al., 1991). Once extracted, otoliths were placed in plastic vials containing ultrapure (Milli-Q) water and left at least overnight. This cleaned the otoliths and helped separate any connective tissue still present. Otoliths were then rinsed in household bleach and triple-rinsed in Milli-Q water. They were dried overnight in a fume hood and stored in glass vials prior to mounting.

Once dry, the left-side otoliths were mounted in two-part epoxy resin. If the left otolith could not be extracted, the right otolith was used. Latex moulds were first half-filled with the epoxy resin and allowed to set overnight. A small amount of resin was then placed in the moulds to aid otolith positioning. The otoliths were then placed in the moulds in identical orientation. The moulds were then filled with epoxy resin and left to harden for around 4 days.

When the resin had hardened, the otoliths were examined under a dissecting microscope to locate the nucleus of the otolith, which was marked using a scalpel. The mounted otoliths were then cut into 0.5 mm sections using a Buehler Isomet low-speed diamond saw. Excess epoxy resin was trimmed using scissors and the sections were mounted on a small piece of glass microscope slide using cyanoacrylate glue (Loctite™). This step prevented the otoliths from breaking during polishing. The piece of glass and otolith were then mounted on a glass slide using Crystal Bond™ thermal mountant. The otoliths were then polished using 400-1200 grit waterproof silicon carbide paper until the nucleus was clearly visible.

The sections were finally mounted on a microscope slide for laser ablation. Approximately 12 otoliths were mounted on one slide. The prepared slides were sonicated for 5 minutes in a sonicating bath in an acid-washed plastic container containing Milli-Q water to remove any surface contamination. Finally, the prepared slides were rinsed in Milli-Q water and dried overnight in a fume hood. They were then stored in resealable plastic bags until ablation.

2.4 Otolith laser ablation

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was chosen over traditional tagging and marking methods because it provides an easily accessible record of the fishes' entire life history. For this study, laser ablation was chosen over liquid ICP-MS because fine spatial resolution was required (detecting differences between nucleus and edge regions). Although several studies have used whole dissolved otoliths as a tracer of fish life history (e.g. Campana et al., 2000 & 2007), solutions carry a risk of contamination and loss of volatile elements (Durrant, 1999). Liquid ICP-MS is insensitive to changes in otolith microchemistry lasting for short periods of up to a year (Campana et al., 2007), whereas LA-ICP-MS allows measurement of small portions of the otolith and therefore short periods of the fishes' life.

Otoliths were ablated using a New Wave Research UP-213 Laser Ablation System with a 213 nm neodymium yttrium aluminium garnet (Nd-YAG) laser. Ablated material was carried using a mixture of helium and argon gas to a Perkin Elmer DRCII ELAN 6000 inductively coupled mass spectrometer. Settings are given in Table 2.1. Isotopes analysed included magnesium (^{25}Mg), aluminium (^{27}Al), calcium (^{42}Ca and ^{43}Ca), manganese (^{55}Mn), copper (^{65}Cu), zinc (^{66}Zn), nickel (^{62}Ni), rubidium (^{85}Rb), strontium (^{88}Sr) and barium (^{138}Ba) (Table 2.1). ^{60}Ni was measured in initial samples to confirm that interference of other elements was producing the ^{62}Ni signal. ^{75}As substituted ^{60}Ni for later analyses, but could not be used in statistical tests because it was not measured in all samples. These isotopes were chosen because they were unlikely to give a false signal because of interference of other elements. The carrier gas used was a mixture of Ar and He, which gives higher sensitivity than using Ar alone (Günther et al., 1999).

Table 2.1 Settings used in laser ablation inductively coupled plasma mass spectrometry analysis of koi carp and goldfish otoliths.

Parameter	Value
Analytes	^{10}B, ^{25}Mg, ^{27}Al, ^{42}Ca (internal standard), ^{43}Ca, ^{55}Mn, ^{62}Ni, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{137}Ba
Sweeps/reading	5
Readings/replicate	155
Replicates	1
Estimated reading	0.845 s
Scan mode	Peak hopping
MCA channels	1
Dwell time per AMU	10 ms

The following start-up protocol was used. The laser was warmed up by firing with the shutter closed until consistent power readings were attained, for 30 to 60 minutes. This was done at 60% laser power, with a repetition rate of 10 Hz and a spot size of 60 µm. The power was recorded each day to ensure comparable power readings between days. The sample chamber was flushed with helium and argon for several minutes in order to clear oxygen from the connecting tubes.

The ICP-MS was optimised before each session, first using a liquid standard, then using a laser ablation standard. The liquid standard contained 2 ppm Sc, 2 ppm Ga, 20 ppb Te, 40 ppb Rh and 20 ppb Lu in Milli-Q water and nitric acid. Optimisation using the laser involved ablating a line scan on the reference material. For the line scan, settings used were 70% laser power, 5 Hz, 50 µm spot size, and 5 µm s^{-1} scanning speed. NIST SRM (National Institute of Standards and Technology Standard Reference Material) 612 was used as a standard for all analyses. The NIST 612 element concentrations published in Pearce et al. (1997) were used in this study.

Laser settings used are given in Table 2.2. Once the laser was optimised, two spots were ablated using the NIST 612 standard. Then, two spots on each otolith were ablated. One spot was ablated at the nucleus of the otolith, representing larval and juvenile growth. Another spot was ablated as close to the edge as possible, representing recent growth. Background element concentrations were measured for 60 s prior to each ablation by analysing a gas blank (firing the laser with the shutter closed). After all otoliths on the slide had been sampled, another two spots on the NIST 612 reference material were ablated, to measure instrument drift during the session. The sample chamber was purged for at least 10 minutes after opening it to introduce new samples, as it was found that background levels of some elements took several minutes to stabilise.

Table 2.2 Laser power, spot size, repetition rate, and laser dwell time used for ablation of koi carp otoliths, goldfish otoliths, and NIST 612.

	Laser power	Spot size	Repetition rate	Laser dwell time
NIST 612	60%	60 µm	10 Hz	60 s
Otoliths	50%	50 µm	5 Hz	40 s

Data were selected and reduced using GLITTER (GEMOC Laser ICP-MS Total Trace Element Reduction) (Van Achterbergh et al., 2001). To calculate element concentrations, counts were standardised to the stoichiometric abundance of CaO in $CaCO_3$, which is 56.03%. ^{42}Ca was used as an internal standard. Ablation of

the NIST 612 reference material at the beginning and end of ablation allowed the results to be linearly interpolated for each session, to account for instrument drift. The final output given by GLITTER reports element concentrations in ppm (weight for weight) corrected from the isotopic counts given by the ICP-MS using natural isotopic abundances. The mean concentration for each laser spot is given. Minimum detection limits (MDL) were calculated by GLITTER at the 99% confidence interval using background readings and Poisson counting statistics. The following formula was used:

$$MDL = 2.3 \times \sqrt{2B}$$

where *B* is the total counts in the background interval (van Achtenberg et al., 2001).

GLITTER allows the user to visually select which portion of the laser ablation signal is used in calculating element concentrations. The first few seconds of ablation were not used in order to avoid any surface contamination of the otolith.

2.5 Water chemistry

Water samples were taken from Lake Waikare, Lake Waahi, Lake Whangape, the Whangamarino River, and the Waikato River at Aka Aka and Rangiriri (Figure 2.1) on one occasion in January 2008. Three samples were taken from each site using 15 ml syringes and then filtered using Millipore 0.45 μm filter units. Latex gloves were worn during sampling and whenever the water sampling equipment was handled. Samples were stored on ice for transport, and then they were preserved with nitric acid (2% of sample volume). Samples were then analysed using inductively coupled plasma mass spectrometry (ICP-MS) to determine elemental concentrations. A rinse solution of acidified Type 1 water was run for 30 seconds between each sample, and a flush was carried out every eight samples. Merck standards XXI and IV were used as a quality control to check for instrument drift.

2.6 Statistical analyses

Data were ln transformed in order to meet the assumptions of ANOVA and linear discriminant function analysis. Zero readings were substituted with 0.1 in order to obtain a log value. Cases (otolith spots) were excluded if one or more element concentrations fell outside three standard deviations of the mean.

Analyses of variance, post-hoc Tukey HSD tests, Spearman rank correlations, nonparametric Kruskal-Wallis tests, and stepwise linear discriminant function analyses (DFA) were carried out using STATISTICA, version 8 (Statsoft, Inc., 2007). Kruskal-Wallis tests were used to test differences in elemental concentrations between sites if the assumptions of parametric ANOVA were not met. This was assessed using Levene's test for homogeneity of variances. A linear stepwise DFA was used, with a priori classification probabilities proportional to group sizes. Mg was not used in the DFA because transformed data for this element did not meet assumptions of normality.

Chapter 3. Results

3.1 Water chemistry

Elemental concentrations in water samples were significantly different between locations (Wilks' lambda<0.001; F=2473; p<0.001. Zn, Rb and As concentrations were higher at the Waikato River at Rangiriri and Aka Aka than other sites (Table 3.1b). Mean As concentrations were significantly different between all sites, except between Lake Waikare and the Whangamarino River, and between the Waikato River at Rangiriri and the Waikato River at Aka Aka (Tukey's HSD test, Appendix 1). Mean Rb concentrations were not significantly different between the Waikato River at Rangiriri and the Waikato River at Aka Aka, but were significantly different between all other sites (Tukey's HSD test, Appendix 1). Significant differences were found in mean water Zn concentrations between the two river sites, but not between any other sites (Tukey's HSD test, Appendix 1). Mean Al levels in Lake Waikare and the Whangamarino River were significantly higher than all other sites (Table 3.1a; Tukey's HSD test, Appendix 1).

Lake Waahi water samples had higher concentrations of B, Mg, Ca, Ni, Sr and Ba than the other sites sampled (Tables 3.1a & 3.1b). Lake Waahi B levels were approximately twice those of Lake Whangape, the site with the second highest B levels (Table 3.1a). Significant differences in mean B concentrations were evident between all sites (Tukey's HSD test, Appendix 1). Water samples from Lake Waahi had significantly higher concentrations of Sr than all other sites, and Sr differences were significantly different between all sites (Table 3.1b; Tukey's HSD test, Appendix 1).

The concentrations of many elements in water samples were correlated; the elements As, Ni and Ba had the most statistically significant correlations with other elements (Spearman's rank correlation, Table 3.2). Rb was negatively correlated with Sr and Ba, while Sr and Ba were highly positively correlated (Spearman's rank correlation, Table 3.2). Ca was positively correlated with Mg,

Sr, Ba and Ni, and Mg was positively correlated with Ni, Sr and Ba (Spearman's rank correlation, Table 3.2).

Table 3.1a Mean untransformed elemental concentrations in ppb of B, Mg, Al, Ca, Mn, and Ni in water for each site. *N*=3 for each location.

	B		Mg		Al		Ca		Mn		Ni	
Location	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lake Waahi	691.8	13.72	10596	422.9	19.59	2.34	11930	377.2	1.58	0.58	1.40	0.02
Lake Waikare	165.0	1.81	1246	24.0	314.58	27.99	2091	43.4	7.60	1.19	0.59	0.13
Lake Whangape	358.7	14.04	3397	15.1	44.72	11.36	8167	85.4	6.51	1.95	1.27	0.07
Waikato River at Aka Aka	258.9	6.57	3101	23.5	15.98	0.93	2900	42.4	56.39	4.31	0.50	0.01
Waikato River at Rangiriri	287.0	7.76	3037	34.0	7.55	0.23	2618	54.9	16.78	0.60	0.20	0.01
Whangamarino River	77.4	2.27	4383	21.4	63.48	8.18	3000	16.9	743.01	6.55	0.72	0.17

Table 3.1b Mean untransformed elemental concentrations in ppb of Cu, Zn, As, Rb, Sr and Ba in water for each site. *N*=3 for each location.

	Cu		Zn		As		Rb		Sr		Ba	
Location	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lake Waahi	0.71	0.07	2.23	1.10	0.94	0.09	6.64	0.09	429.9	5.72	43.3	0.57
Lake Waikare	0.83	0.11	1.17	0.78	4.49	0.10	7.78	0.03	42.8	0.21	16.8	0.13
Lake Whangape	0.62	0.04	1.77	1.54	2.31	0.01	3.70	0.03	175.7	3.42	19.4	0.33
Waikato River at Aka Aka	0.47	0.07	3.62	0.36	23.19	0.28	15.61	0.24	45.5	0.53	14.6	0.57
Waikato River at Rangiriri	0.12	0.02	0.41	0.07	23.98	0.40	15.83	0.16	38.1	0.31	13.0	0.11
Whangamarino River	0.52	0.10	3.83	4.16	4.40	0.16	4.90	0.03	68.3	0.40	21.1	1.15

Table 3.2 Spearman rank order correlation coefficients between elemental concentrations in water samples. Correlations significant at the p<0.05 level are shown in bold italics.

	B	Mg	Al	Ca	Mn	Ni	Cu	Zn	Rb	As	Sr
Mg	0.370										
Al	***-0.482***	-0.022									
Ca	***0.598***	***0.940***	-0.084								
Mn	***-0.781***	-0.143	-0.032	-0.364							
Ni	0.441	***0.742***	0.397	***0.808***	***-0.562***						
Cu	0.115	0.121	***0.705***	0.174	***-0.583***	***0.668***					
Zn	-0.156	0.337	0.096	0.255	0.195	0.304	0.168				
Rb	-0.102	***-0.552***	***-0.556***	***-0.604***	0.261	***-0.746***	-0.408	-0.158			
As	-0.426	***-0.709***	-0.414	***-0.779***	***0.614***	***-0.934***	***-0.635***	-0.187	***0.773***		
Sr	***0.484***	***0.858***	0.183	***0.917***	-0.455	***0.909***	0.420	0.410	***-0.701***	***-0.889***	
Ba	0.201	***0.810***	***0.474***	***0.763***	-0.352	***0.895***	***0.587***	0.261	***-0.713***	***-0.886***	***0.868***

A forward stepwise discriminant function analysis (DFA) was performed using the $\log_{10}$ transformed water chemistry data (STATISTICA Version 8, Statsoft, Inc., 2007). The elements Mg, Al, Ca, Mn, Zn, Rb, As, Sr and Ba were included in the model, which was statistically significant (Wilks' Lambda<0.001; approx. $F_{55,12}$=3879; p<0.001) and was able to correctly predict the collection site of 100% of the samples (Table 3.3). Three canonical root functions were created. Plots of standardised canonical scores showed that the sites were clearly differentiated (Figure 3.1).

Correlations between standardised canonical root scores and the $\log_{10}$ elemental concentrations in water were measured using a Spearman's rank correlation analysis (Table 3.4). This showed that Root 1 was associated with increasing Rb and As, and decreasing Al, Ni, and Ba (Table 3.4). Root 2 was positively correlated with Mn and Zn and negatively correlated with Al and Cu (Table 3.4). Root 3 was positively correlated with Mn and As and negatively correlated with B, Ni, and Cu (Table 3.4).

Table 3.3 Observed and predicted classifications of water samples using stepwise DFA, based on the elemental concentrations of Al, Mn, Zn, Rb, Sr, and Ba. All classifications were 100% correct.

	Predicted classification					
Site	Lake Waahi	Lake Waikare	Lake Whangape	Waikato River at Aka Aka	Waikato River at Rangiriri	Whangamarino River
Lake Waahi	3	0	0	0	0	0
Lake Waikare	0	3	0	0	0	0
Lake Whangape	0	0	3	0	0	0
Waikato River at Aka Aka	0	0	0	3	0	0
Waikato River at Rangiriri	0	0	0	0	3	0
Whangamarino River	0	0	0	0	0	3
Total	3	3	3	3	3	3

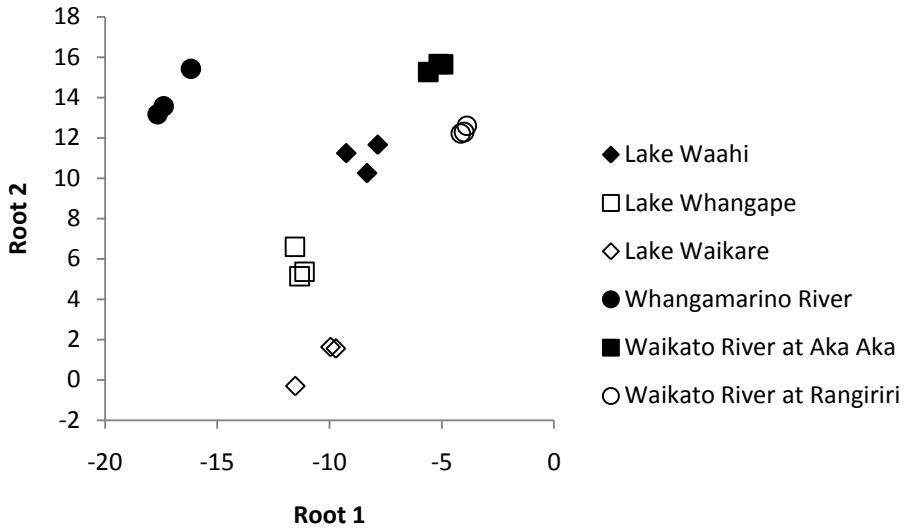

Figure 3.1 Standardised canonical Root 1 scores versus Root 2 scores for each site created in DFA of untransformed water elemental concentrations.

Table 3.4 Spearman's rank correlations between elements in water at six Waikato sites, and standardised canonical root scores. Bold italics indicate significant correlations at the *p*=0.05 level.

	Root 1	Root 2	Root 3
B	0.401	-0.271	***-0.593***
Mg	-0.342	0.257	-0.090
Al	***-0.847***	***-0.505***	-0.199
Ca	-0.265	0.079	-0.257
Mn	-0.141	***0.707***	***0.882***
Ni	***-0.515***	-0.315	***-0.579***
Cu	-0.447	***-0.645***	***-0.771***
Zn	-0.139	***0.501***	-0.015
Rb	***0.777***	0.304	0.125
As	***0.527***	0.401	***0.571***
Sr	-0.418	-0.073	-0.424
Ba	***-0.635***	-0.185	-0.362

3.2 Fork length statistics

A total of 14 goldfish and 108 koi carp were caught from Lake Hakanoa, Lake Waahi, Lake Whangape, Lake Waikare, Opuatia Stream, the Maramarua River, the Whangamarino River, Pungarehu Stream, and the Waikato River at Aka Aka and Rangiriri (Table 3.5). Of the 108 carp caught, 91 were longer than 140 mm F.

L. and 17 were shorter than 140 mm F. L. (Table 3.5). This length was used to distinguish adults from juveniles, as fish shorter than 140 mm F. L. are likely less than 1 year old and sexually immature (Tempero et al., 2006). Fish shorter than 140 mm F. L. are hereafter referred to as young of the year (YOY) and fish longer than this length are hereafter referred to as adult. The mean length for young-of-the-year (YOY) carp was 74.2 mm ± 34.0 SD (Table 3.5). The mean length for adult carp was 420.5 mm ± 87.5 SD (Table 3.5).

Table 3.5 Fork length descriptive statistics for koi carp sorted by site.

			Fork length (mm)			
Capture site	Adult/ YOY	*n*	Mean	Max	Min	SD
Lake Hakanoa	Adult	1	475.0	475	475	0.0
Lake Waahi	Adult	9	489.9	569	405	50.9
Lake Waikare	Adult	14	437.5	514	330	46.9
	YOY	6	64.8	78	47	11.1
Lake Whangape	Adult	14	425.6	510	302	49.0
Maramarua River	YOY	2	126.0	133	119	8.1
Opuatia Stream	Adult	8	334.5	535	250	108.9
Pungarehu Stream	Adult	8	255.8	320	223	39.7
	YOY	7	50.3	73	33	14.0
Waikato River at Aka Aka	Adult	11	459.7	591	389	50.5
Waikato River at Rangiriri	Adult	16	482.8	620	410	50.2
Whangamarino River	Adult	10	378.9	443	271	45.0
	YOY	2	134.0	137	131	3.5
Adult total		91	420.5	514	271	87.5
YOY total		17	74.2	137	33	34.0
Koi carp total		108	365.9	514	47	150.3

A total of 14 goldfish were caught from the Maramarua River, the Whangamarino River, and Pungarehu Stream (Table 3.6). The mean length for goldfish was 126 mm ± 50.5 SD (Table 3.6).

Table 3.6 Fork length descriptive statistics for goldfish sorted by site.

		Fork length (mm)			
Capture site	*n*	Mean	Max	Min	SD
Maramarua River	4	112.9	168	69	42.3
Pungarehu Stream	4	141.8	211	85	61.4
Whangamarino River	6	124.4	196	67	49.2
Total	14	126.1	211	67	50.5

3.3 Minimum detection limits

The percentage of readings above detection limits varied between elements (Table 3.7). ^{75}As and ^{60}Ni were not used in further analyses as they were not measured in all samples.

Table 3.7 Percentage of readings above 99% minimum detection limits (MDL) for isotopes measured in koi carp and goldfish otoliths.

Isotope	^{10}B	^{25}Mg	^{27}Al	^{55}Mn	^{60}Ni	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba
% above MDL	31	96	21	53	75	22	34	37	67	45	100	100

3.4 Otolith elemental concentrations

Mean elemental concentrations in the edges and nuclei of koi carp otoliths are given in Tables 3.8a and 3.8b. Mean elemental concentrations in goldfish otolith nuclei and edges are given in Table 3.10. Rb was the least abundant element in carp and goldfish otoliths, with concentrations below 1 ppm (Tables 3.8a, 3.8b & 3.9). Mn was typically present at levels below 4 ppm, and Mg and Ba were found at concentrations between 10 ppm and 150 ppm (Tables 3.8a, 3.8b & 3.9). Sr was the most abundant trace element measured, with concentrations ranging between 1100 and 1900 ppm (Tables 3.8a, 3.8b & 3.9). Other elements (B, Al Ni, Cu, and As) were also measured but were not used in further analyses because they were not either not measured in all samples or not found at levels above

detection limits in the majority of samples. Ca was also measured, but was used as an internal standard, so cannot be used to distinguish between habitats.

Mean concentrations of Mg, Mn, Zn, Sr and Ba in the edges of koi carp otoliths were significantly different between at least two sites (Tukey's unequal *N* HSD test, Appendix 2). Otolith edges of koi carp caught at Pungarehu Stream showed higher concentrations of all elements compared to other sites, except Sr (Tables 3.8a & 3.8b). Tukey's unequal *N* HSD tests showed that mean otolith edge Mg, Mn, Zn and Ba concentrations of koi carp caught at Pungarehu Stream were significantly different to at least one other site (Appendix 2). Mean Mg concentrations in otolith edges of koi carp caught at Pungarehu Stream were significantly different to those caught at the Waikato River at Aka Aka, the Waikato River at Rangiriri, the Whangamarino River and Lake Whangape (Tukey's unequal *N* HSD test, Appendix 2). Mean Mn concentrations in otolith edges of koi carp caught at Pungarehu Stream were significantly different to those caught from Lake Waikare (Tukey's unequal *N* HSD test, Appendix 2).

Otolith edges of koi carp caught from Lake Hakanoa, Lake Waahi and the Maramarua River had the highest Sr concentrations (Tables 3.8a & 3.8b). Mean Sr concentrations in otolith edges of koi carp caught at Lake Waahi were significantly different to those caught from the Waikato River at Aka Aka and Rangiriri, Opuatia Stream, and Lake Whangape (Tukey's unequal *N* HSD test, Appendix 2). Mean Mg concentrations in otolith edges of fish caught at Lake Waikare were high but variable; there were no significant differences in Mg between otolith edges of koi carp from Lake Waikare and those from other sites (Tables 3.8a & 3.8b; Tukey's unequal *N* HSD test, Appendix 2).

Of the elements measured in the otolith edges of koi carp, Ba concentrations showed the most significant differences between sites (Tukey's unequal *N* HSD test, Appendix 2). Ba concentrations in otolith edges of koi carp caught at Pungarehu Stream were higher than other sites, and were significantly different to Ba concentrations in the otolith edges of fish caught at the Waikato River at Aka Aka and Rangiriri, Opuatia Stream, the Whangamarino River, Lake Waahi,

and Lake Whangape (Tables 3.8a & 3.8b; Tukey's unequal *N* HSD test, Appendix 2). Mean Ba concentrations in otolith edges of fish caught at Opuatia Stream were significantly different to those caught at Pungarehu Stream, Lake Waikare and the Whangamarino River (Tukey's unequal *N* HSD; Appendix 2).

No significant differences in elemental concentrations were found between otolith edges of koi carp from Lake Waikare and fish from the Whangamarino River (Tukey's unequal *N* HSD test, Appendix 2). Mn was the only element showing significant differences between otolith edges of fish caught at Pungarehu Stream and fish caught at Lake Waikare (Tukey's unequal *N* HSD test, Appendix 2).

No significant differences were found in Mg, Zn and Rb concentrations in koi carp otolith nuclei between sites (Tukey's unequal *N* HSD test, Appendix 3). Ba concentrations showed the most significant differences between sites in koi carp otolith nuclei (Tukey's unequal *N* HSD test, Appendix 3). Otolith nuclei of koi carp from Pungarehu Stream showed the highest Ba concentrations, similar to otolith edges from this location (Tables 3.8a & 3.8b). The Ba concentrations in otoliths from Pungarehu Stream were significantly different to those from Opuatia Stream and Lake Waahi (Tukey's unequal *N* HSD test, Appendix 3). Ba concentrations in the otolith nuclei of fish caught at Opuatia Stream were significantly different to those from Pungarehu Stream, the Waikato River at Rangiriri, Lake Waikare, Lake Whangape and the Whangamarino River.

Mean Mn concentrations in the otolith nuclei of koi carp caught at the Waikato River at Rangiriri were significantly different to those caught at Lake Waikare and Pungarehu Stream (Tukey's unequal *N* HSD test, Appendix 3). Significant differences in mean Sr concentrations were evident only between otolith nuclei in koi carp from the Waikato River at Rangiriri and Lake Whangape (Tukey's unequal *N* HSD test, Appendix 3). Overall, otolith element concentrations were variable between and within sites (Tables 3.8a & 3.8b).

Table 3.8a Mean untransformed elemental concentrations (ppm) in koi carp otoliths from Lake Hakanoa, Lake Waahi, Lake Waikare, Lake Whangape, Maramarua River, Opuatia Stream, Pungarehu Stream, and the Waikato River at Aka Aka, sorted by capture location, adult or young-of-the-year (YOY), and otolith nucleus or edge.

Capture Location	Adult or YOY	Nucleus/ Edge	*n*	Mg		Mn		Zn		Rb		Sr		Ba	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lake Hakanoa	Adult	Nucleus	1	16.73	0.00	1.13	0.00	5.11	0.00	0.18	0.00	1706.0	0.0	53.66	0.00
		Edge	1	7.12	0.00	0.48	0.00	2.73	0.00	0.21	0.00	2026.0	0.0	63.98	0.00
Lake Waahi	Adult	Nucleus	11	26.35	8.97	0.57	0.50	2.72	5.22	0.29	0.21	1423.8	462.7	23.85	18.23
		Edge	11	14.83	10.78	0.28	0.17	7.31	12.48	0.34	0.18	1776.8	464.9	24.73	10.48
Lake Waikare	Adult	Nucleus	14	23.32	13.10	0.89	1.23	2.12	2.58	0.32	0.19	1382.3	184.1	49.15	15.92
		Edge	14	17.46	15.21	0.29	0.37	3.73	8.43	0.44	0.40	1338.1	197.3	31.94	14.00
	YOY	Nucleus	6	185.10	386.80	0.38	0.34	3.59	2.22	0.22	0.23	1247.2	432.7	42.34	25.82
		Edge	6	182.14	333.14	0.37	0.29	7.20	8.39	0.27	0.22	1235.8	433.5	51.57	31.55
Lake Whangape	Adult	Nucleus	14	21.47	6.04	0.73	0.67	2.46	5.75	0.27	0.14	1182.9	261.6	32.06	22.33
		Edge	14	17.74	11.69	0.32	0.28	6.76	18.79	0.28	0.15	1195.8	268.6	23.59	14.94
Maramarua River	YOY	Nucleus	2	28.35	21.62	0.43	0.31	9.38	7.08	0.25	0.26	1898.9	575.3	33.93	4.08
		Edge	2	8.14	1.42	0.53	0.60	9.69	13.56	0.21	0.14	1821.7	123.0	48.20	4.79
Opuatia Stream	Adult	Nucleus	8	37.97	11.96	0.73	0.46	2.35	2.30	0.33	0.16	1240.6	268.3	17.58	19.48
		Edge	8	22.90	10.74	0.36	0.34	1.14	1.34	0.30	0.14	1152.7	221.5	13.65	12.37
Pungarehu Stream	Adult	Nucleus	8	52.87	23.54	3.13	6.28	3.37	4.12	0.50	0.21	1264.9	156.9	146.52	202.17
		Edge	8	22.35	18.82	0.50	0.42	7.00	9.24	0.57	0.31	1331.1	142.5	50.20	11.09
	YOY	Nucleus	7	25.93	13.20	0.15	0.13	1.54	1.81	0.30	0.15	1353.8	105.1	59.22	18.41
		Edge	7	84.96	58.47	1.79	1.24	16.48	11.20	0.24	0.12	1428.2	107.1	101.31	39.31
Waikato River at Aka Aka	Adult	Nucleus	11	25.83	11.12	0.91	0.58	1.41	1.65	0.44	0.28	1116.2	201.6	25.73	13.70
		Edge	11	11.19	3.39	0.30	0.17	1.37	1.26	0.50	0.25	1038.6	187.8	17.04	6.51

Table 3.8b Mean untransformed elemental concentrations (ppm) in koi carp otoliths from the Waikato River at Rangiriri and the Whangamarino River, sorted by capture location, adult or young-of-the-year (YOY), and otolith nucleus or edge.

		Nucleus/		Mg		Mn		Zn		Rb		Sr		Ba	
Capture Location	Adult or YOY	Edge	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Waikato River at Rangiriri	Adult	Nucleus	16	30.60	14.90	2.50	3.07	2.84	3.00	0.41	0.19	1532.9	342.2	60.09	27.41
		Edge	16	56.62	173.30	0.73	1.69	1.26	1.98	0.56	0.25	1142.7	370.4	24.90	12.32
Whangamarino River	Adult	Nucleus	10	35.97	34.93	0.65	0.69	1.61	1.43	0.38	0.18	1288.7	165.5	41.17	20.44
		Edge	10	13.42	5.48	0.18	0.15	1.44	2.24	0.41	0.27	1288.2	167.2	33.86	18.62
	YOY	Nucleus	2	71.43	10.53	1.78	0.07	14.68	1.85	0.32	0.16	1499.3	507.7	35.46	3.91
		Edge	2	16.28	2.18	1.51	0.16	1.35	1.76	0.38	0.01	1297.4	1.0	51.88	3.13

Table 3.9 Mean untransformed elemental concentrations (ppm) in goldfish otoliths, sorted by capture location and otolith nucleus or edge.

			Mg		Mn		Zn		Rb		Sr		Ba	
Capture site	Nucleus/Edge	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Maramarua River	Nucleus	4	30.18	11.37	7.65	5.91	4.49	5.95	0.30	0.24	1518.8	617.3	63.65	53.05
	Edge	4	15.48	11.03	2.49	2.47	7.62	8.90	0.41	0.13	1756.6	280.0	85.79	41.92
Pungarehu Stream	Nucleus	4	28.32	8.08	1.09	0.67	3.09	2.39	0.35	0.28	1353.3	237.4	50.51	16.57
	Edge	4	17.18	15.85	0.92	0.37	5.95	4.37	0.37	0.24	1369.4	106.7	84.47	31.77
Whangamarino River	Nucleus	6	25.70	21.59	5.10	4.65	3.20	3.61	0.22	0.13	1466.7	196.7	69.42	42.29
	Edge	6	22.07	24.73	4.51	6.62	11.91	12.39	0.40	0.19	1417.8	283.1	74.84	36.36

3.5 Comparisons of elemental concentrations

Significant differences were found between capture sites in the mean otolith elemental concentrations of koi carp. Data were transformed using a natural log (ln) transformation to normalise data for ANOVAs and post-hoc Tukey tests. Untransformed data were used for nonparametric ANOVAs and Spearman rank correlations.

3.5.1 Adult koi carp otolith edges

Significant differences between sites were found in the mean Rb, Sr and Ba concentrations of adult koi carp otolith edges (Table 3.10). Lake Hakanoa was excluded from ANOVA comparisons between adult koi carp nucleus and edge element concentrations because of sample size limitations (Table 3.1). Variances were homogeneous for all elements except Ba (Levene's test for homogeneity, p= 0.02, Appendix 4). A nonparametric ANOVA showed significant differences between locations in median Ba concentrations in otolith edges (Kruskal-Wallis test, $H(7,N=84)=28.9$; $p=0.0002$). Nonparametric Spearman's rank order correlations were calculated for each element (Table 3.11). Significant positive correlations were found between Zn and Mg, between Zn and Sr, and between Sr and Ba (Table 3.12).

Table 3.10 Univariate ANOVA results comparing mean ln-transformed concentrations (ppm) of Mg, Mn, Zn, Rb, Sr and Ba between sites in otolith edges of adult koi carp (df=7,1). Bold italics show significant differences between sites for that element.

	SS	MS	F	p
Mg	2.697	0.385	1.353	0.238
Mn	7.447	1.064	1.049	0.405
Zn	41.994	5.999	1.972	0.070
Rb	***7.393***	***1.056***	***2.453***	***0.025***
Sr	***1.458***	***0.208***	***4.690***	***<0.001***
Ba	***13.324***	***1.903***	***4.801***	***<0.001***

Table 3.11 Spearman's rank order correlation coefficients for untransformed elemental concentrations (ppm) in otolith edges of adult koi carp. Correlations significant at the *p*=0.05 level are shown in bold italics.

	Mg	Mn	Zn	Rb	Sr
Mn	0.149				
Zn	***0.261***	0.191			
Rb	-0.003	0.160	-0.047		
Sr	0.147	-0.061	***0.233***	-0.186	
Ba	0.064	0.112	0.088	0.086	***0.417***

3.5.2 Adult koi carp otolith nuclei

Analysis of variance showed significant differences between sites in mean concentrations of Mg, Mn, Sr and Ba in adult koi carp otolith nuclei (Table 3.12). Variances were not homogeneous for Mg, Zn, Sr or Ba (Levene's test for homogeneity, p=0.001, p=0.001, p=0.004, 0.001, Appendix 5). A nonparametric ANOVA showed significant differences in median Ba concentrations (Kruskal-Wallis test, $H(7,N=90)=32.1$; $p<0.0001$) and median Sr concentrations (Kruskal-Wallis test, $H(7,N=90)=22.0$; $p<0.0025$) in otolith nuclei between sites. Significant differences were also found in median otolith nucleus Mg concentrations (Kruskal-Wallis test, $H(7,N=90)=18.5$; $p=0.0101$), but not median Zn concentrations (Kruskal-Wallis test, $p>0.05$). Nonparametric Spearman's rank order correlations were calculated for each element (Table 3.14). Mg was positively correlated with Mn and Zn (Table 3.13). Significant positive correlations were found between between Ba and Sr and Zn and Mn (Table 3.13).

Table 3.12 Univariate ANOVA results comparing mean ln-transformed concentrations (ppm) of Mg, Mn, Zn, Rb, Sr and Ba between sites in otolith nuclei of adult koi carp (df=7,1). Bold italics show significant differences between sites for that element.

	SS	MS	F	p
Mg	***4.832***	***0.690***	***2.933***	***0.009***
Mn	***19.017***	***2.717***	***2.303***	***0.034***
Zn	15.955	2.279	0.963	0.464
Rb	4.369	0.624	1.401	0.216
Sr	***0.938***	***0.134***	***3.314***	***0.004***
Ba	***27.517***	***3.931***	***7.069***	***<0.001***

Table 3.13 Spearman's rank order correlation coefficients for untransformed elemental concentrations (ppm) in otolith nuclei of adult koi carp. Correlations significant at the *p*=0.05 level are shown in bold italics.

	Mg	Mn	Zn	Rb	Sr
Mn	***0.408***				
Zn	***0.500***	***0.345***			
Rb	0.014	0.068	0.087		
Sr	-0.175	-0.032	-0.085	0.062	
Ba	-0.005	0.176	-0.032	0.112	***0.576***

3.5.3 Young-of-the-year koi carp otolith edges

Significant differences between sites were found in mean Mg and Mn concentrations in YOY koi carp otolith edges (Table 3.14). Fewer elements showed significant differences between sites in the otolith edges of YOY koi carp than elements in the edges and nuclei of adult koi carp otoliths (Tables 3.10, 3.12 & 3.14). For YOY carp, only otoliths of fish from Pungarehu Stream and Lake Waikare were analysed because of sample size limitations for the Whangamarino River and the Maramarua River (Table 3.1). Variances of all elements were homogeneous, except Mn and Sr (Levene's test for homogeneity, $p>0.05$, Appendix 6). A non-parametric ANOVA showed significant differences in median Mn concentrations (Kruskal-Wallis test, $H(2,N=12)=6.5$; $p=0.0384$), but not in median Sr concentrations (Kruskal-Wallis test, $p>0.05$). Nonparametric Spearman's rank order correlations (Table 3.15) showed that elements were not as highly correlated in YOY koi carp edges as in adult koi carp otoliths (Tables

3.11 & 3.13), but significant positive correlations were found between Mg and Zn and between Mg and Ba (Table 3.16). Sr and Ba were not correlated in YOY koi carp otolith edges, though they were in adult koi carp otolith nuclei and edges (Tables 3.11, 3.13 & 3.15).

Table 3.14 Univariate ANOVA results comparing mean ln-transformed concentrations (ppm) of Mg, Mn, Zn, Rb, Sr and Ba between sites in otolith edges of YOY koi carp (df=1,1). Bold italics show significant differences between sites for that element.

	SS	MS	F	p
Mg	***3.110***	***1.555***	***5.223***	***0.035***
Mn	***11.349***	***5.674***	***7.445***	***0.015***
Zn	16.770	8.385	2.708	0.126
Rb	0.720	0.360	1.044	0.395
Sr	0.020	0.010	3.968	0.064
Ba	0.993	0.496	3.282	0.091

Table 3.15 Spearman's rank order correlation coefficients for untransformed elemental concentrations (ppm) in otolith edges of YOY koi carp. Correlations significant at the *p*=0.05 level are shown in bold italics.

	Mg	Mn	Zn	Rb	Sr
Mn	0.462				
Zn	***0.932***	0.497			
Rb	-0.032	0.265	0.180		
Sr	-0.063	-0.350	-0.112	-0.434	
Ba	***0.587***	0.371	0.396	-0.342	0.399

3.5.4 Young-of-the-year koi carp otolith nuclei

Significant differences between sites in mean concentrations of Mg and Mn were found in the otolith nuclei of YOY koi carp (Table 3.16). All elements in otolith nuclei of YOY carp had homogeneous variances except Zn and Sr (Levene's test for homogeneity, $p<0.001$, Appendix 7). No significant differences in median Zn or Sr concentrations were found between sites (Kruskal-Wallis test, $p>0.05$). Nonparametric Spearman's rank order correlations (Table 3.17) showed that fewer elements were correlated in YOY koi carp otolith nuclei than in adult koi

carp otoliths or YOY koi carp otolith edges (Tables 3.11, 3.13 & 3.15). Zn and Mg were positively correlated, and Ba and Mn were negatively correlated in YOY koi carp nuclei at a statistically significant level (Table 3.17).

Table 3.16 Univariate ANOVA results comparing mean ln-transformed concentrations (ppm) of Mg, Mn, Zn, Rb, Sr and Ba between sites in otolith nuclei of YOY koi carp (df=1,1). Bold italics show significant differences between sites for that element.

	SS	MS	F	p
Mg	***2.011***	***1.006***	***5.210***	***0.026***
Mn	***11.851***	***5.926***	***7.433***	***0.009***
Zn	20.22	10.11	3.349	0.073
Rb	0.470	0.235	0.410	0.674
Sr	0.013	0.006	0.416	0.670
Ba	0.386	0.193	2.189	0.158

Table 3.17 Spearman's rank order correlation coefficients for untransformed elemental concentrations (ppm) in otolith nuclei of YOY koi carp. Correlations significant at the *p*=0.05 level are shown in bold italics.

	Mg	Mn	Zn	Rb	Sr
Mn	0.332				
Zn	***0.555***	0.421			
Rb	-0.164	-0.268	0.140		
Sr	-0.354	0.197	0.232	0.146	
Ba	-0.437	***-0.563***	-0.389	0.487	0.108

3.5.5 Goldfish otoliths

No significant differences were shown between sites in any of the elements measured in goldfish otolith edges or nuclei (ANOVA; $p>0.05$, Appendices 8 & 9). Variances were homogeneous for all elements measured at the edges of goldfish otoliths, but the variances of Rb and Sr were not homogeneous between sites in the nuclei of goldfish otoliths (Levene's test for homogeneity, $p>0.05$). Nonparametric tests showed no significant differences between sites for median Rb or Sr concentrations in goldfish otolith nuclei (Kruskal-Wallis test, $p>0.05$). All

three sites where goldfish were caught, Maramarua River, Whangamarino River and Pungarehu Stream, were included in this analysis.

3.5.6 Comparison between goldfish and koi carp otolith edge element concentrations

Otolith edge concentrations of koi carp and goldfish caught from the Whangamarino River, the Maramarua River and Pungarehu Stream were compared. No significant differences were found between species in means of any element. No significant differences were found between species caught at any other location for any other element (Tukey's unequal *N* HSD test, $p>0.05$).

3.6 Discriminant function analysis

A forward stepwise discriminant function analysis was carried out using the otolith edge element concentrations (STATISTICA Version 8, Statsoft Inc., 2007). A natural log (ln) transformation was applied to the data prior to analysis in order to improve normality. Some pairs of adjacent sites were combined, as the model could not accurately distinguish between them. The Waikato River at Aka Aka and the Waikato River at Rangiriri were combined to form the group Waikato River, and Pungarehu Stream and Lake Waikare were combined to form the group Pungarehu and Waikare. The Whangamarino River and Lake Whangape were excluded from the model because the capture site of fish from these locations could not be accurately predicted using otolith edge element concentrations.

The final model included four groups: Opuatia Stream, Pungarehu Stream and Lake Waikare, Waikato River, and Lake Waahi. The model incorporated the elements (in order of inclusion) Ba, Sr, and Rb, and had high discriminatory power (Wilks' Lambda=0.360; $F_{9,158}=9.180$; $p<0.001$). Otolith edge elemental concentrations were classified to their capture site with an overall accuracy of 75% (Table 3.18). Fish caught at Pungarehu Stream and the Waikato River at Aka

Aka were classified most accurately, i.e. 92% and 91% respectively (Table 3.18). Fish caught from the Waikato River at Rangiriri and Opuatia Stream were classified the least accurately, with only 53% and 63% of cases classified correctly (Table 3.18).

Table 3.18 Capture site of adult and YOY koi carp compared to predicted classification, with total fish from each site and the percentage of fish that were classified correctly. Predicted classifications were calculated using stepwise DFA of Ba, Sr, and Rb concentrations in koi carp otolith edges.

	Predicted classification using otolith edge					
Capture site	Opuatia Stream	Pungarehu and Waikare	Waikato River	Lake Waahi	Total	% correct
Waikato River at Aka Aka	1	0	10	0	11	91
Waikato River at Rangiriri	1	6	8	0	15	53
Pungarehu Stream	0	11	1	0	12	92
Lake Waikare	2	12	1	0	15	80
Opuatia Stream	5	1	2	0	8	63
Lake Waahi	0	1	2	7	10	70
Total	9	31	24	7	71	75
Waikato River combined	2	6	18	0	24	75
Pungarehu & Waikare combined	2	23	2	0	27	85

Because four groups were included in the DFA, three canonical root functions were produced (Equation Table 3.1). The respective eigenvalues for the first, second, and third roots were 0.732, 0.497, and 0.072.

Equation Table 3.1 Standardised canonical root functions created in stepwise discriminant function analysis of ln transformed element concentrations of koi carp otolith edges. Element concentrations in ppm.

$$Root\ 1 = 0.869612(\ln Ba) + 0.2826(\ln Sr) - 0.011902(\ln Rb)$$

$$Root\ 2 = -0.596866(\ln Ba) + 0.90692(\ln Sr) - 0.413585(\ln Rb)$$

$$Root\ 3 = -0.137516(\ln Ba) + 0.468968(\ln Sr) + 0.920437(\ln Rb)$$

A nonparametric Spearman's rank correlation analysis was carried out on the root scores and the otolith edge element concentrations (Table 3.19). Root 1 was positively correlated with Sr and Ba at a statistically significant level (Table 3.19). Root 2 was negatively correlated with Ba and Rb, and Root 3 was positively correlated with Rb at a statistically significant level (Table 3.19).

Table 3.19 Spearman rank order correlation coefficients between canonical root scores and ln element concentrations (ppm) in edges of koi carp otoliths. Bold italics indicate significant correlations at the *p*=0.05 level.

	Root 1	Root 2	Root 3
Rb	-0.083	***-0.548***	***0.974***
Sr	***0.528***	0.135	-0.153
Ba	***0.994***	***-0.667***	-0.155

Otolith edges were assigned to locations using the classification functions created in the DFA (Equation Table 3.2). The classification functions (Equation Table 3.2) were then used to classify otolith nucleus concentrations of all adult (Table 3.20) and YOY (Table 3.21) koi carp.

Equation Table 3.2 Classification functions created using stepwise discriminant function analysis of ln transformed edge element concentrations of koi carp otoliths. Elemental concentrations in ppm.

$$Opuatia = -645.447 - 11.666(\ln Ba) + 188.913(\ln Sr) + 1.847(\ln Rb)$$

$$Pungarehu\ \&\ Waikare = -659.621 - 14.038(\ln Ba) + 191.364(\ln Sr) - 0.023(\ln Rb)$$

$$Waikato\ River = -675.044 - 10.1(\ln Ba) + 192.201(\ln Sr) + 0.938(\ln Rb)$$

$$Waahi = -720.972 - 12.603(\ln Ba) + 199.484(\ln Sr) + 0.652(\ln Rb)$$

In 45% of adult koi carp, the otolith nucleus signature matched the site of capture (Table 3.20). Sixteen of the 20 adult koi carp caught at Pungarehu Stream and Lake Waikare had nucleus signatures matching the site where they were captured (Table 3.20). Four adult koi carp caught at Opuatia Stream had nucleus signatures corresponding to Opuatia Stream, and the remaining four fish matched the Waikato River (2 fish) and Pungarehu Stream and Lake Waikare (2 fish) (Table 3.20).

In many cases, the predicted nucleus classification of adult koi carp did not match the capture site. For example, of the 27 adult koi carp caught from the Waikato River, 16 had nucleus signatures characteristic of the Pungarehu Stream and Lake Waikare signature, and only seven had otolith nucleus signatures matching the Waikato River (Table 3.20). However, carp caught from the Waikato River at Rangiriri had a greater proportion of nuclei classified to Pungarehu and Waikare (13 of 16, or 81%) than carp caught at the Waikato River at Aka Aka (3 of 11, or 27%) (Table 3.20). Most carp caught from the Waikato River at Aka Aka (55%) had nucleus signatures matching the Waikato River (Table 3.20). Of the 11 koi carp with fork lengths greater than 140 mm caught at Lake Waahi, only three had nucleus signatures that corresponded to their site of capture (Table 3.20). Only half the fish caught from Opuatia Stream had nucleus signatures match their capture site (Table 3.20).

Table 3.20 Capture site of koi carp > 140 mm F. L. compared to classification of otolith nucleus. The nucleus classification was predicted using the discriminant functions created using otolith edge signatures. % match = the percentage of fish where the capture site matches the nucleus classification.

Capture site	Predicted classification using otolith nucleus				Total	% match
	Opuatia Stream	Pungarehu and Waikare	Waikato River	Lake Waahi		
Waikato River at Aka Aka	1	3	6	1	11	55
Waikato River at Rangiriri	0	13	1	2	16	6
Pungarehu Stream	0	3	3	0	6	50
Lake Waikare	0	13	1	0	14	93
Opuatia Stream	4	2	2	0	8	50
Lake Waahi	3	2	3	3	11	27
Total	8	36	16	6	66	45
Waikato River sites combined	1	16	7	3	27	26
Pungarehu & Waikare combined	0	16	4	0	20	80

All otolith nucleus elemental signatures of koi carp < 140 mm F. L. were classified their site of capture, which was Lake Waikare and Pungarehu Stream (Table 3.21).

Table 3.21 Capture site of koi carp < 140 mm F. L. compared to classification of otolith nucleus. The nucleus classification was predicted using the discriminant functions created using otolith edge signatures. % match = the percentage of fish where the capture site matches the nucleus classification.

Capture site	Predicted classification using otolith nucleus				Total	% match
	Waikato River	Opuatia Stream	Pungarehu and Waikare	Lake Waahi		
Pungarehu Stream	0	0	7	0	7	100
Lake Waikare	0	0	5	0	5	100
Total	0	0	12	0	12	100
Pungarehu & Waikare combined	0	0	12	0	12	100

Chapter 4. Discussion

4.1 Water chemistry

Otolith microchemistry is thought to be largely determined by water chemistry (Campana, 1999). Therefore, significant differences in water chemistry between locations indicate possible differences in otolith microchemistry of fish. In water samples, significant differences between sites were found in the concentrations of many elements. Water samples from Lake Waahi had higher concentrations of B, Mg, Ca, Ni, Sr and Ba than other sites, and water samples from the Waikato River at Rangiriri and Aka Aka had higher Cu, As and Rb than other sites. Concentrations of Mg, Ca, Ni, Sr, and Ba were positively correlated in water samples.

Concentrations of As were high in water from the Waikato River at Aka Aka and Rangiriri compared to other locations sampled. This is likely due to geothermal activities in the upper catchment of the Waikato River. The concentrations of As measured in water samples from the Waikato River at Rangiriri and the Waikato River at Aka Aka were similar to published values (Beard, 2007). Arsenic concentrations were 23 ppb ± 0.3 SD at Aka Aka, and 24 ppb ± 0.4 SD at Rangiriri in the present study. Beard (2007) measured As concentrations of 17 ppb at the Waikato River at Tuakau Bridge, near Aka Aka, and 18 ppb at the Waikato River at Mercer, near Rangiriri. Measured As concentrations were similar to the maximum As levels measured by Beard (2007) of 25 ppb at Mercer and 26 ppb at Tuakau. Beard's (2007) values represent the mean of monthly samples taken over one year, whereas samples were taken on one occasion in the present study, in January 2008. The high As concentrations measured in the present study may be due to seasonal fluctuations in As caused by variation in precipitation and tributary inputs.

B concentrations measured in water samples from the Waikato River at Rangiriri and the Waikato River at Aka Aka are within the ranges measured by Beard

(2007) at the Waikato River at Mercer, near Rangiriri, and the Waikato River at Tuakau, near Aka Aka. The concentrations measured in the present study (259 ppb at Aka Aka and 287 ppb at Rangiriri) are slightly higher than the yearly mean calculated by Beard (2007) of 210 ppb for Mercer and Tuakau. However, these higher results are likely due to seasonal fluctuations and are below the maximum B concentrations measured by Beard (2007) of 340 ppb at Mercer and 360 ppb at Tuakau.

Ca values measured in the Waikato River were lower than those reported in previous studies. Lam (1981) measured yearly mean Ca concentrations of 6500 ppb ± 600 SE at the Waikato River at Rangiriri, and 6900 ppb ± 700 SE at the Waikato River at Tuakau, near Aka Aka. Similar Ca concentrations to those measured by Lam (1981) were measured by Environment Waikato in 1995 (Environment Waikato, unpublished data). In the present study, the concentration of Ca was 2618 ppb ± 54.9 SD at the Waikato River at Rangiriri and 2900 ppb ± 42.4 SD at the Waikato River at Aka Aka.

Mg levels measured in the present study were 3.0 ppm ± 0.3 SD at the Waikato River at Rangiriri, and 3.1 ppm ± 0.2 SD at the Waikato River at Aka Aka. These concentrations are similar to those published by Lam (1981) of 2.5 ppm ± 0.7 SE at the Waikato River at Rangiriri, and 2.3 ± 0.6 SE at the Waikato River at Tuakau. Seasonal fluctuations in Ca, Mg and B levels may have caused discrepancies between published and measured values, as water elemental concentrations were only measured on one occasion in the present study.

Discriminant function analyses (DFA) were carried out on the water sample elemental concentrations and the koi carp otolith edge elemental concentrations. The correlations between elemental concentrations and root scores created in the DFAs were analysed in order to ascertain which elements were most important in discriminating locations. The water root scores and the koi carp otolith root scores were not correlated with the same elements, indicating that different elements were needed to distinguish between sites for water samples and otoliths. In the DFA of water samples, the first root was

positively correlated with As and Rb, and negatively correlated with Al, Ni and Ba. In the DFA of koi carp otolith edges, the first root was positively correlated with Ba, Sr, Mg and Zn, and negatively correlated with Rb. The second root in the water DFA was positively correlated with Mn and Zn, and negatively correlated with Al and Cu. Mn was not included in the DFA created using koi carp otolith edges. Sr was positively correlated with all three root scores in the otolith edge DFA, while Sr not correlated with the root scores of in the water DFA at a statistically significant level. The differences in correlations between root scores and otolith concentrations are likely due to metabolic regulation of otolith element concentration.

Ba and Sr will substitute Ca in the $CaCO_3$ lattice because they are also divalent metals (Speer, 1983), and otolith concentrations of Ba and Sr are usually proportional to water concentrations (Farrell & Campana, 1996; Bath et al., 2000; Crook et al., 2006). Increased Sr in water was found by de Vries et al (2005) to facilitate the uptake of Ba into juvenile black bream *Acanthopagrus butcheri* otoliths. Sr incorporation into the otolith matrix is thought to introduce deformities in the crystal structure, allowing Ba to be more easily incorporated into the otolith (de Vries et al., 2005).

4.2 Fork length statistics

The mean fork length for adult koi carp was 420.5 mm ± 87.5 SD, which corresponds to an age of approximately 5 years (Tempero et al., 2006). A similar mean fork length (430 mm ± 98.6 SD) was reported in a recent study of koi carp in the Waikato River (Osborne et al., in press). If young-of-the-year (YOY) fish are included in the mean length, the average length is 365.9 mm ± 150.3 SD, which corresponds to an age of approximately 4 years (Tempero et al., 2006).

4.3 Elemental concentrations in koi carp and goldfish otoliths

Significant differences were found between locations in the concentrations of Rb, Sr and Ba in koi carp otolith edges in the lower Waikato area. This indicated that otolith chemistry could be used as a natural tag to differentiate between locations and track fish movement. In the nuclei of adult koi carp, significant differences between sites were found in Mg, Mn, and Sr and Ba. Significant differences between Pungarehu Stream and Lake Waikare were found in Mg and Mn concentrations in YOY koi carp otolith edges and nuclei.

Concentrations of many elements in water samples and otoliths were correlated. Correlations between Sr and Ba concentrations were found in water samples and adult koi carp otolith edges and nuclei. A correlation between Sr and Ba was also found in the otoliths of chum salmon (*Oncorhynchus keta*) by Arai and Hirata (2006).

No significant differences were found between sites in element concentrations of either goldfish otolith nuclei or edges. A possible reason for this is that the sites where goldfish were caught, the Whangamarino River, the Maramarua River and Pungarehu Stream, were too close together, sharing water sources. The Whangamarino River and the Maramarua River both drain the Whangamarino Wetland, which receives water from Lake Waikare via Pungarehu Stream. The sample numbers of goldfish may also have been too small to detect any differences. Though the study of movement in this species using otolith microchemistry seems feasible, larger sample sizes and a wider range of sampling areas are required.

Elemental concentrations in koi carp and goldfish were similar, and no significant differences were found in any element concentrations between goldfish and koi carp caught in the Whangamarino River, Maramarua River and Pungarehu Stream. Although uptake of elements into otoliths is species specific, probably due to metabolic differences, similarities in otolith elemental concentrations have been found between co-occurring species (Hamer & Jenkins, 2007). The otolith elemental concentrations of koi carp and goldfish from a broader range of

locations need to be compared before any definitive conclusions can be made regarding differences in otolith element incorporation between these species.

4.4 Identification of spawning sites

A forward stepwise discriminant function analysis (DFA) was carried out using the otolith edge signatures of koi carp. Concentrations of Ba, Sr and Rb were used to classify otoliths to one of four sites: Waikato River, Lake Waahi, Opuatia Stream, or Pungarehu Stream and Lake Waikare combined. The capture site of 75% of koi carp was accurately predicted using elemental concentrations from the otolith edges. The capture site of fish from the Waikato River at Rangiriri was predicted the least accurately, with only 53% classified correctly. Fish caught from Rangiriri were often misclassified to the nearby area of Pungarehu Stream and Lake Waikare, suggesting that fish caught at Rangiriri may have been recent migrants into the Waikato River.

The discriminant function created using koi carp otolith edge elemental concentrations was used as a training set to classify the elemental concentrations of koi carp otolith nuclei. The nucleus of the otolith is laid down in the larval and juvenile stages and reflects the natal habitat of the fish (Campana et al., 2000). The classifications of otolith nucleus signatures of all koi carp under 140 mm F. L. caught at Pungarehu Stream and Lake Waikare matched the capture site, indicating that these fish had not yet dispersed. The classification of the otolith nucleus matched the site of capture for 16 of 20 adult fish caught at Lake Waikare and Pungarehu Stream. These fish therefore had not dispersed, or had returned to their natal site after dispersal. Similarly, four of the eight koi carp caught at Opuatia Stream had otolith nucleus signatures corresponding to their capture site.

Sixteen of the 27 adult koi carp caught at the Waikato River had otolith nucleus elemental signatures corresponding to Lake Waikare and Pungarehu Stream. Carp caught from the Waikato River at Rangiriri had a greater proportion of nuclei classified to Pungarehu and Waikare (13 of 16, or 81%) than carp caught at

the Waikato River at Aka Aka (3 of 11, or 27%). Six of the 11 adult carp caught at the Waikato River at Aka Aka had nucleus sites matching the Waikato River, suggesting these fish originated from a Waikato River site. Koi carp spawning has been observed at Aka Aka, and the results of this study suggest that this spawning, or spawning at other sites in the Waikato River, has been successful. Carp caught at the Waikato River at Rangiriri, however, are likely to have originated from Lake Waikare and Pungarehu Stream. Therefore, Lake Waikare and Pungarehu Stream are likely providing a source of koi carp recruits both locally and for the Waikato River.

Otolith nuclei of koi carp from Lake Waahi were classified to a range of sites, with four fish classified to Lake Waahi, four fish classified to the Waikato River, and three fish classified to Opuatia Stream. This suggests that fish caught at Lake Waahi originated from a range of locations in the lower Waikato area.

In summary, Lake Waikare and Pungarehu Stream appear to be sources of koi carp recruits. Koi carp from this area appear to be moving to other locations, including the Waikato River at Rangiriri and Lake Waahi. The Waikato River also appears to provide koi carp recruits, though only for the Waikato River at Aka Aka.

Osborne et al. (in press) and Stuart and Jones (2006b) used external tags and mark-recapture methods to estimate distance moved by koi carp in the Waikato region and the Murray-Darling Basin in Australia. Stuart and Jones (2006b) concluded that 80% of tagged koi carp moved less than 5 km, and Osborne et al. (in press) estimated that 86% of tagged koi carp moved less than 5 km. While the distance moved by koi carp was not quantified in the present study, it was found that 45% of adult koi carp, and 100% of YOY koi carp, had either not left their natal location or had returned to it. The majority of koi carp caught at Lake Waikare and Pungarehu Stream appeared to have originated there. In contrast, few of the fish caught at Lake Waahi or the Waikato River at Rangiriri appeared to have originated there, as they had otolith nucleus signatures matching other locations.

Movement of carp is likely to have been underestimated by previous tagging studies carried out in New Zealand and Australia, such as those by Osborne et al. (in press) and Stuart and Jones (2006b). Preliminary results from a radio tagging study of koi carp in the lower Waikato region indicate that of 12 fish tagged, 10 migrated an average of 44 km (males) and 30 km (females) during 148- 179 days at liberty (Daniel, A., personal communication, 18 June 2008). While mark-recapture studies measure net movement by fish, they may underestimate total movement, as repetitive movements by fish cannot be quantified. This limitation also applies to the present study, as only the edge and nucleus of the otolith were analysed. Any movements made by the fish between the periods when the nucleus and the otolith edge were laid down will not be quantified. This could be remedied in future studies by analysing more spots across the otolith surface.

Otolith edge elemental signatures from fish caught in Lake Whangape and the Whangamarino Wetland were not included in the DFA because they could not be accurately classified to capture locations. Koi carp captured at these locations may have recently moved there from somewhere else, meaning their otoliths had not yet incorporated the local chemical signature. It is also possible that the chemical composition of the water at these locations is not distinct enough to create a discernable difference in otolith elemental signatures. The Whangamarino River and Whangamarino Wetland receive flood waters from the Waikato River, which are diverted into Lake Waikare and into the wetland via Pungarehu Stream. This was reflected in the element edge concentrations of the koi carp caught in Pungarehu Stream, Lake Waikare and the Whangamarino River; no significant differences in any element were found between fish from the Whangamarino River and the other two locations.

4.5 Limitations of discriminant function analysis

Although the DFA of koi carp otoliths used Rb, Sr and Ba, other authors have differentiated between natal locations in freshwater using different elements. Crook and Gillanders (2006) used Mn, Sr, and Ba, while Brazner et al. (2007) used

only Ba and Sr. In the present study, because a forward stepwise DFA was used, elements were only added to the model if they had sufficient discriminatory power, indicating that these four elements were necessary to differentiate between locations.

Mg varied widely within sites in the lower Waikato region, and no significant differences were found between locations in the otolith edge concentrations of Mg in adult koi carp. Mg was not included in the DFA of koi carp otolith edges. Crook and Gillanders (2006) also found that Mg concentrations in otoliths of koi carp did not differ significantly between sites in the Murray-Darling Basin, and did not include Mg in the DFA of otolith elemental concentrations. Mg is likely highly metabolically regulated (Campana et al., 2000) and is therefore likely to vary within locations.

Although Mn was found above detection limits in 53% of the otolith spots, it was not useful in distinguishing between locations in the lower Waikato region and was not included in the stepwise DFA. The only significant differences in Mn otolith edge concentrations were between otoliths from Pungarehu Stream, and otoliths from the Waikato River at Rangiriri and Lake Waikare. Mn was negatively correlated to water concentrations, possibly due to metabolic regulation. Elsdon and Gillanders (2003) also found that Mn levels in otoliths were not related to water concentrations. However, otolith Mn concentrations have been used successfully to distinguish natal areas of koi carp populations in the Murray-Darling Basin (Crook & Gillanders, 2006) and natal streams of rainbow trout in Lake Rotorua and Lake Rotoiti (Riceman, 2007).

Ba and Sr were useful in distinguishing between sites in this study and are widely used in otolith microchemistry studies to differentiate between locations (Thresher, 1999). Ba and Sr are thought to be fairly rigidly incorporated in the otolith and are not sensitive to differences in sample preparation (Campana et al., 2000). As well as this, Ba concentrations in otoliths are proportional to concentrations in water, making Ba effective in discriminating between locations (Farrell & Campana, 1996; Bath et al., 2000; Crook et al., 2006).

4.6 How assumptions affect interpretation of otolith microchemistry

Elsdon and Gillanders (2004) outlined some assumptions involved in tracing fish life history using otolith microchemistry. One assumption is that the fish being examined has remained long enough in the environment in question to pick up the ambient chemical signature in its otolith; this is likely to require some hours or days (Elsdon & Gillanders, 2004). As such, otolith microchemistry studies may not be suitable for very fast moving fish or rapidly changing environments (Elsdon & Gillanders, 2004). Koi carp are capable of moving long distances in a short time, moving as far as 64 km in 244 days (Daniel, A., personal communication, 18 June, 2008), so fish may pass through environments without picking up the chemical signature. An effect of this is that otoliths of fish that have very recently moved will not immediately show the chemical signature of the new habitat. The laser spot size used to analyse koi carp and goldfish otoliths, 50 µm, was found to correspond to 34 days of otolith growth in wild pacific Chum salmon (Sanborn & Telmer, 2003). Assuming similar otolith accretion rates in koi carp and Chum salmon, koi carp would need to have spent at least 34 days in an environment before the chemical signature of that environment was incorporated in the otolith. In this study, it was found that the laser could not be fired at the very edge of the otolith, as this often caused the otolith to fracture. Because of this, we can estimate that a fish would need to spend at least approximately 60 days in a particular location for the otolith to reflect the chemical signature of the new environment, rather than the previous environment. This may be the reason why only 75% of fish edge otolith signatures were classified correctly using the DFA. It is possible that misclassified fish had recently moved to their capture location, and that their otoliths still had the previous environment's chemical signature.

Another assumption is that otolith microchemistry is not affected by ontogenetic factors (Elsdon & Gillanders, 2004). Little is known about ontogenetic effects on the uptake of elements into otoliths. Fowler et al. (1995), after rearing Atlantic croaker *Micropogonias undulatus* from hatching to 71 days under different

environmental conditions, found that ontogenetic effects interacted with temperature and salinity. Chittaro et al. (2006) also found ontogenetic differences in otolith chemistry between early life stages. Here, differences were found between the otolith element concentrations of juvenile and embryo otoliths. Elevated levels of Mn have been found in the otolith nuclei of clupeid fish compared to the otolith edges, suggesting an ontogenetic effect on Mn concentration (Brophy et al., 2004). No evidence was found, however, for any ontogenetic changes in otolith element concentration in later life stages, e.g. between young adult fish and older adult fish, or between juveniles and adults. No influence of ontogeny on Ba and Sr levels could be found in adult fish by Elsdon and Gillanders (2005). In carp, the juvenile phase begins at 20-25 mm (Vilizzi & Walker, 1998), and no fish below this length were caught in the present study. Judging from current knowledge, we can surmise that no ontogenetic effects are likely to affect the analysis of juvenile and adult koi carp otolith signatures, though this should be confirmed in the lab by future researchers on this topic.

We also assumed in this study that the chemical signatures of the environments where fish were caught have not changed during the life span of the fish (approximately five years). Long term monitoring of water chemistry would be needed to test this assumption. However, the success of previous studies indicates that this assumption is reasonable. Aquatic environments are relatively buffered from high-frequency fluctuations in water chemistry compared to the terrestrial environment (Campana & Thorrold, 2001). As well as this, otoliths are separated from the outside environment by the membranes separating endolymph and blood, and those separating blood and water, further buffering any short-term fluctuations (Campana and Thorrold, 2001). We can therefore reasonably assume that elemental signatures from otolith nuclei, laid down when the fish was young, can be compared to elemental signatures at the edge of the otolith. However, Gillanders (2002) found significant differences in juvenile estuarine fish otolith chemistry among recruitment years, and recommends building up a library of otolith chemical signatures for each location

using fish of different year classes. This was not feasible in the short period of this study, but is recommended for future studies.

4.7 Comparison with previous studies

The percentage of correct predicted classifications in the discriminant function analysis (DFA) in this study (75%) is lower than reported in previous studies. It is likely that this percentage could be improved by including the otolith signatures of YOY fish from more locations. The otolith signatures of young fish would be more likely to be characteristic of that location, compared to the otolith chemical signatures of adults, who may be recent migrants. Crook and Gillanders (2006) used YOY fish to create the distinctive chemical signatures of the natal areas, and were able to successfully predict capture sites with a high degree of accuracy using maximum likelihood analysis. Riceman (2008) used the otolith chemical signatures of juvenile rainbow trout to create a discriminant function which was then used as a training set to classify otolith nucleus signatures and to identify natal streams of adult trout. The classification functions predicted capture location of the juvenile trout with an overall accuracy of 91% (Riceman, 2008).

It is possible, however, to accurately classify fish using the otolith chemical signatures of adult fish. Brazner et al. (2004) used otoliths of adult yellow perch (*Perca flavescens*) from Lake Superior, USA, in a discriminant function analysis. Using only Sr and Ba, the capture site of 86% of fish from four locations were classified correctly (Brazner et al., 2004).

The design of this experiment could have been improved by using equal sample sizes among locations. This could have been achieved in this study by randomly excluding otoliths from some samples, but this was not done, as the process of acquiring otolith chemistry data was time consuming.

Ca is used as a standard in LA-ICP-MS, but not all elements correlate well with Ca because of differences in elemental fractionation (Longerich et al., 1996). Most of the elements measured in the present study (Mg, Mn, Rb, Sr, Ba) display

similar ablation behaviour to Ca and can be measured using Ca as a standard. Zn, however, which was used in the DFA of koi carp otolith elemental concentrations, had the poorest correlation with Ca of all elements measured by Longerich et al. (1996). Longerich et al. (1996) used a 266 nm laser to achieve these results. It has since been found that 216 nm lasers, which were used in the present study, can significantly reduce the incidence of elemental fractionation compared 266 nm lasers (Günther et al., 2003).

Other structures, such as fin rays and scales, can also be used in otolith microchemistry studies with some success (e.g. Clarke et al., 2007); however, these structures take up elements from the blood, which can vary in ion concentration more than the endolymph (Payan et al., 1999). Scales and fin rays, unlike otoliths, can be reabsorbed by the fish (Clarke et al., 2007), meaning important information could be lost. Thus, when it is not imperative to sample without harming the fish, otoliths are likely to be the better option for LA-ICP-MS studies.

4.8 Conclusions

This study used otolith microchemistry to track the movement of koi carp (*Cyprinus carpio*) in the lower Waikato region. Discriminant function analysis was used to differentiate between otolith edge signatures of fish caught from Lake Waahi, Opuatia Stream, the Waikato River, and Lake Waikare and Pungarehu Stream combined. The DFA was able to classify fish to their capture sites with 75% accuracy. The classification functions created in the DFA were then used as a training set to classify the elemental signatures of koi carp otolith nuclei. This showed that while most koi carp caught at the Waikato River at Aka Aka and Lake Waikare originated from the site they were captured, most fish caught at the Waikato River at Rangiriri and Lake Waahi are likely to have originated from elsewhere.

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Appendices

Appendix 1 Pairwise comparisons of untransformed mean elemental concentrations (ppm) in water samples (Tukey's HSD test, df=12).

B

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***<0.001***			
Whangamarino River	***<0.001***	***<0.001***	***<0.001***		
Waikato River at Aka Aka	***<0.001***	***<0.001***	***<0.001***	***<0.001***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***0.005***

Mg

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***<0.001***			
Whangamarino River	***<0.001***	***<0.001***	***<0.001***		
Waikato River at Aka Aka	***<0.001***	***0.001***	***<0.001***	***<0.001***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	0.755

Appendix 1 (Continued) Comparisons of mean untransformed elemental concentrations (ppm) in water samples (Tukey's HSD test, df=12).

Al

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***<0.001***			
Whangamarino River	***<0.001***	***0.042***	***<0.001***		
Waikato River at Aka Aka	0.446	***<0.001***	***<0.001***	***<0.001***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

Ca

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***<0.001***			
Whangamarino River	***<0.001***	***<0.001***	***<0.001***		
Waikato River at Aka Aka	***<0.001***	***<0.001***	***<0.001***	0.320	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

Mn

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***0.879***			
Whangamarino River	***<0.001***	***<0.001***	***<0.001***		
Waikato River at Aka Aka	***<0.001***	***<0.001***	***<0.001***	***<0.001***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***0.004***	***<0.001***	***<0.001***

Appendix 1 (Continued) Comparison of mean untransformed elemental concentrations (ppm) in water samples (Tukey's HSD test, df=12).

Ni

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	0.932				
Lake Waikare	***<0.001***	***<0.001***			
Whangamarino River	***<0.001***	***0.002***	0.451		
Waikato River at Aka Aka	***<0.001***	***<0.001***	0.640	***0.043***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

Cu

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	0.808				
Lake Waikare	0.717	0.155			
Whangamarino River	0.099	0.554	***0.009***		
Waikato River at Aka Aka	***0.018***	0.138	***0.002***	0.897	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

Zn

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	0.987				
Lake Waikare	0.736	0.969			
Whangamarino River	0.998	0.902	0.511		
Waikato River at Aka Aka	0.920	0.620	0.247	0.991	
Waikato River at Rangiriri	0.147	0.359	0.772	0.077	***0.030***

Appendix 1 (continued) Comparisons of mean untransformed elemental concentrations (ppm) in water samples (Tukey's HSD test, df=12).

As

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***<0.001***			
Whangamarino River	***<0.001***	***<0.001***	0.992		
Waikato River at Aka Aka	***<0.001***	***<0.001***	***<0.001***	***<0.001***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	0.913

Rb

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***<0.001***			
Whangamarino River	***<0.001***	***<0.001***	***<0.001***		
Waikato River at Aka Aka	***<0.001***	***<0.001***	***<0.001***	***<0.001***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	0.602

Sr

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***<0.001***			
Whangamarino River	***<0.001***	***<0.001***	***<0.001***		
Waikato River at Aka Aka	***<0.001***	***<0.001***	***<0.001***	***<0.001***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

Appendix 1 (continued) Comparisons of mean untransformed elemental concentrations (ppm) in water samples (Tukey's HSD test, df=12).

Ba

	Lake Waahi	Lake Whangape	Lake Waikare	Whangamarino River	Waikato River at Aka Aka
Lake Whangape	***<0.001***				
Lake Waikare	***<0.001***	***0.001***			
Whangamarino River	***<0.001***	***0.043***	***<0.001***		
Waikato River at Aka Aka	***<0.001***	***<0.001***	***0.001***	***<0.001***	
Waikato River at Rangiriri	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***0.004***

Appendix 2 Comparisons of mean ln transformed elemental concentrations (ppm) in koi carp otolith edges (Tukey's unqeual *N* HSD test, df=87).

Mg

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	0.505						
Pungarehu Stream	***0.002***	0.801					
Waikato River at Rangiriri	1.000	0.689	***0.004***				
Lake Waahi	0.987	0.935	0.065	0.999			
Lake Waikare	0.411	1.000	0.456	0.425	0.946		
Whangamarino River	0.996	0.873	***0.016***	1.000	1.000	0.825	
Lake Whangape	0.980	0.939	***0.031***	0.998	1.000	0.922	1.000

Mn

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	1.000						
Pungarehu Stream	0.285	0.554					
Waikato River at Rangiriri	1.000	1.000	0.117				
Lake Waahi	1.000	1.000	0.268	1.000			
Lake Waikare	0.992	0.993	***0.030***	0.999	0.998		
Whangamarino River	0.999	0.999	0.058	1.000	1.000	1.000	
Lake Whangape	1.000	1.000	0.145	1.000	1.000	0.999	1.000

Appendix 2 (Continued) Comparisons of mean ln transformed elemental concentrations (ppm) in koi carp otolith edges (Tukey's unqeual *N* HSD test, df=95).

Zn

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	1.000						
Pungarehu Stream	0.300	0.279					
Waikato River at Rangiriri	0.914	0.997	***0.008***				
Lake Waahi	0.860	0.735	0.992	0.177			
Lake Waikare	1.000	1.000	0.341	0.713	0.922		
Whangamarino River	0.968	1.000	***0.017***	1.000	0.267	0.912	
Lake Whangape	0.993	1.000	***0.034***	1.000	0.392	0.972	1.000

Rb

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	0.784						
Pungarehu Stream	0.999	0.960					
Waikato River at Rangiriri	1.000	0.565	0.975				
Lake Waahi	0.841	1.000	0.984	0.612			
Lake Waikare	0.555	1.000	0.848	0.140	1.000		
Whangamarino River	0.921	1.000	0.996	0.683	1.000	0.997	
Lake Whangape	0.360	1.000	0.666	0.129	0.998	1.000	0.971

Appendix 2 (Continued) Comparisons of mean ln transformed elemental concentrations (ppm) in koi carp otolith edges (Tukey's unqeual *N* HSD test, df=95).

Sr

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	0.971						
Pungarehu Stream	***0.022***	0.560					
Waikato River at Rangiriri	0.683	1.000	0.639				
Lake Waahi	***<0.001***	***0.007***	0.409	***0.008***			
Lake Waikare	***0.020***	0.546	1.000	0.479	0.425		
Whangamarino River	0.154	0.916	0.993	0.976	0.097	0.992	
Lake Whangape	0.748	1.000	0.566	1.000	***0.006***	0.548	0.957

Ba

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	0.648						
Pungarehu Stream	***<0.001***	***<0.001***					
Waikato River at Rangiriri	0.803	0.059	***0.004***				
Lake Waahi	0.924	0.096	***0.006***	1.000			
Lake Waikare	0.091	***0.002***	0.202	0.750	0.795		
Whangamarino River	0.327	***0.009***	***0.044***	0.993	0.982	0.998	
Lake Whangape	0.998	0.306	***<0.001***	0.984	0.999	0.285	0.687

Appendix 3 Comparisons of mean ln transformed elemental concentrations (ppm) in koi carp otolith nuclei (Tukey's unqeual *N* HSD test, df=96).

Mg

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	0.699						
Pungarehu Stream	0.814	1.000					
Waikato River at Rangiriri	0.994	0.970	0.994				
Lake Waahi	1.000	0.829	0.925	1.000			
Lake Waikare	1.000	0.532	0.531	0.901	0.999		
Whangmarino	0.966	0.993	1.000	1.000	0.994	0.856	
Whangape	0.999	0.374	0.334	0.790	0.990	1.000	0.688

Mn

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	1.000						
Pungarehu Stream	0.373	0.676					
Waikato River at Rangiriri	0.826	0.861	***0.004***				
Lake Waahi	0.910	0.982	0.984	0.128			
Lake Waikare	0.743	0.920	0.999	***0.008***	1.000		
Whangamarino River	0.999	1.000	0.715	0.394	0.998	0.963	
Whangape	0.996	1.000	0.766	0.218	1.000	0.975	1.000

Appendix 3 (Continued) Comparisons of mean ln transformed elemental concentrations (ppm) in koi carp otolith nuclei (Tukey's unqeual *N* HSD test, df=93).

Zn

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	0.951						
Pungarehu Stream	1.000	0.970					
Waikato River at Rangiriri	0.973	1.000	0.977				
Lake Waahi	1.000	0.969	1.000	0.985			
Whangamarino River	1.000	0.979	1.000	0.975	1.000		
Whangape	0.980	1.000	0.988	1.000	0.990	0.993	
Wha.	1.000	0.793	0.998	0.706	0.999	0.995	0.813

Rb

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	1.000						
Pungarehu Stream	1.000	1.000					
Waikato River at Rangiriri	1.000	0.994	0.998				
Lake Waahi	0.728	0.967	0.791	0.404			
Lake Waikare	0.989	1.000	0.992	0.730	0.994		
Whangamarino River	1.000	1.000	1.000	1.000	0.632	0.965	
Whangape	0.938	0.998	0.942	0.569	1.000	1.000	0.865

Appendix 3 (Continued) Comparisons of mean ln transformed elemental concentrations (ppm) in koi carp otolith nuclei (Tukey's unqeual *N* HSD test, df=93).

Sr

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	0.973						
Pungarehu Stream	0.528	0.998					
Waikato River at Rangiriri	***0.006***	0.342	0.478				
Lake Waahi	0.175	0.930	0.998	0.927			
Lake Waikare	0.108	0.863	0.982	0.930	1.000		
Whangamarino River	0.432	0.994	1.000	0.633	1.000	0.995	
Whangape	0.998	1.000	0.846	***0.013***	0.521	0.234	0.797

Ba

	Waikato River at Aka Aka	Opuatia Stream	Pungarehu Stream	Waikato River at Rangiriri	Lake Waahi	Lake Waikare	Whangamarino River
Opuatia Stream	0.090						
Pungarehu Stream	0.065	***<0.001***					
Waikato River at Rangiriri	0.065	***<0.001***	1.000				
Lake Waahi	0.998	0.290	***0.010***	***0.010***			
Lake Waikare	0.206	***<0.001***	0.999	0.999	***0.044***		
Whangamarino River	0.902	***0.003***	0.626	0.628	0.539	0.906	
Whangape	0.999	***0.027***	0.140	0.111	0.939	0.345	0.993

Appendix 4 Levene's test for homogeneity of variances for ln transformed otolith edge elemental concentrations of adult koi carp. Bold italics show significant results (non-homogeneous variances) at the *p*=0.05 level.

	MS Effect	MS Error	F	P
Mg	0.172	0.111	1.553	0.162
Mn	0.177	0.324	0.546	0.797
Zn	0.809	0.773	1.047	0.406
Rb	0.111	0.164	0.679	0.690
Sr	0.026	0.014	1.772	0.105
Ba	***0.376***	***0.146***	***2.582***	***0.019***

Appendix 5 Levene's test for homogeneity of variances for ln transformed otolith nucleus elemental concentrations of adult koi carp. Bold italics show significant results (non-homogeneous variances) at the *p*=0.05 level.

	MS Effect	MS Error	F	p
Mg	***0.264***	***0.066***	***3.997***	***0.001***
Mn	0.490	0.364	1.348	0.239
Zn	***1.763***	***0.520***	***3.391***	***0.003***
Rb	0.287	0.215	1.339	0.243
Sr	***0.032***	***0.014***	***2.249***	***0.038***
Ba	***0.913***	***0.153***	***5.989***	***<0.001***

Appendix 6 Levene's test for homogeneity of variances for ln transformed otolith edge elemental concentrations of YOY koi carp. Bold italics show significant results (non-homogeneous variances) at the *p*=0.05 level.

	MS Effect	MS Error	F	p
Mg	0.124	0.093	1.324	0.319
Mn	***0.760***	***0.148***	***5.121***	***0.037***
Zn	2.326	0.647	3.594	0.077
Rb	0.223	0.065	3.419	0.084
Sr	***0.003***	***0.000***	***6.738***	***0.019***
Ba	0.061	0.042	1.459	0.288

Appendix 7 Levene's test for homogeneity of variances for ln transformed otolith elemental concentrations of YOY koi carp. Bold italics show significant results (non-homogeneous variances) at the *p*=0.05 level.

	MS Effect	MS Error	F	p
Mg	0.060	0.066	0.909	0.431
Mn	0.595	0.192	3.092	0.086
Zn	***2.149***	***0.470***	***4.574***	***0.036***
Rb	0.183	0.076	2.392	0.137
Sr	***0.034***	***0.001***	***26.19***	***<0.001***
Ba	0.018	0.037	0.498	0.621

Appendix 8 Univariate ANOVA results comparing mean ln transformed concentrations (ppm) of Mg, Mn, Zn, Rb, Sr and Ba between sites in otolith nuclei of goldfish (df=2,1). Bold italics show significant differences between sites for that element.

	SS	MS	F	p
Mg	50.10	25.05	0.095	0.911
Mn	87.8	43.9	2.254	0.151
Zn	5.15	2.58	0.150	0.862
Rb	0.046	0.023	0.523	0.607
Sr	57969	28985	0.212	0.812
Ba	867.3	433.7	0.262	0.774

Appendix 9 Univariate ANOVA results comparing mean ln transformed concentrations (ppm) of Mg, Mn, Zn, Rb, Sr and Ba between sites in otolith nuclei of goldfish (df=2,1). Bold italics show significant differences between sites for that element.

	SS	MS	F	p
Mg	118.6	59.3	0.156	0.857
Mn	32.0	16.0	0.739	0.500
Zn	95.7	47.8	0.495	0.622
Rb	0.004	0.002	0.051	0.951
Sr	372077	186038	3.053	0.088
Ba	365.9	183.0	0.135	0.875

Appendix 10 Raw untransformed LA-ICP-MS data, sorted by ablation date. Element concentrations are given in ppm. K=koi carp, G=goldfish, c=core, e=edge, Hak=Lake Hakanoa, Opu=Opuatia Stream, Pun=Pungarehu Stream, Aka= Waikato River at Aka Aka, Ran=Waikato River at Rangiriri, Whm=Whangamarino River, Waa=Lake Waahi, Wai=Lake Waikare, Wha=Lake Whangape. **** and <*** indicate zero readings.

Name	Species	Capture site	Core/ Edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight (g)	Length (mm)
WHA008	K	Opu	c	3.54	51.11	4.59	400447	404316	0.49	2.62	2.79	1.61		0.441	1357.82	2.1	5/10	349	250
WHA008	K	Opu	e	<***	30.93	23.06	400447	389701.2	0.21	<***	<***	0.39		0.2	1383.55	7.36	5/10	349	250
WHA009	K	Opu	c	1.07	26.71	4.59	400447	398104.8	0.5	1.39	27.62	1.72		0.252	1126.92	2.48	5/10	409	269
WHA009	K	Opu	e	<***	16.49	3.22	400447	406826.7	0.04	<***	71.86	0.1		0.278	1171.91	5.69	5/10	409	269
WHA011	K	Opu	e	13.45	22.19	0.1	400446.9	403279.7	0.38	****	1.52	1.96		0.497	1175.04	3.4	5/10	338	259
WHA011	K	Opu	c	<***	21.79	2.48	400446.9	399731.6	0.69	****	0.43	2.35		0.347	1648.57	1.64	5/10	338	259
WHA012	K	Opu	e	<***	4.34	5.23	400447	400752.2	0.57	<***	4.51	0.1		0.341	1340.69	36.08	5/10	440	267
WHA012	K	Opu	c	7.87	25.7	3.8	400447	398675.6	0.46	1.28	42.84	1.13		0.558	1267.74	38.85	5/10	440	267
WHA007	K	Opu	c	18.42	37.93	1.32	400446.9	403908.2	0.33	0.91	12.94	0.85		0.481	1438.64	52.73	5/10	406	282
WHA007	K	Opu	e	****	20.68	0.6	400446.9	408089.4	0.135	0.68	6.03	1.05		0.321	1082.18	27.71	5/10	406	282
WAI007	K	Ran	c	10.41	67.24	0.14	400446.9	399908.3	4.31	<***	19.59	1.28		0.399	1225.94	56.04	5/10	1504	410
WAI007	K	Ran	e	24.83	14.04	3.94	400446.9	397642.3	0.51	1.09	12.01	0.1		1.017	1091.48	31.55	5/10	1504	410
WHAi001	K	Ran	c	1.38	33.2	0.59	400446.9	401527.6	1.7	2.42	30.33	4.08		0.367	1105.65	20.6	5/10	4556	620
WHAi001	K	Ran	e	25.51	705.56	2.11	400446.9	402503.1	6.95	<***	24.22	3.81		0.477	114.9	1.46	5/10	4556	620
WHA003	K	Whm	c	3.37	10.59	0.1	400446.9	402840	0.241	1.2	8.5	1.24		0.252	1463.87	41.73	5/10	1155	373
WHA003	K	Whm	e	5.33	10.46	1.04	400446.9	399856.4	0.175	1.16	6.16	1.83		0.859	1403.62	35	5/10	1155	373
wha010	K	Opu	e	21.02	22.32	1.81	400447	407587.4	1.08	<***	0.64	0.25		0.48	1385.41	1.8	31/10	745	324
wha010	K	Opu	c	2.91	45.75	0.1	400447	408271.9	1.65	9.34	5.43	7.92		0.12	1224.6	3.04	31/10	745	324
wai009	K	Ran	c	<***	58.41	32.23	400446.9	400363.3	8.96	1.59	1.94	10.83		0.89	1365.33	72.81	31/10	1599	420

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
wai009	K	Ran	e	<***	22.26	10.31	400446.9	404852.8	0.47	<***	****	1.37		0.6	1399.04	28.59	31/10	1599	420
wai010	K	Ran	c	0	41.3	1.39	400446.9	404866.5	10.43	8.71	18.72	2.41		0.36	1941.23	57.86	31/10	2635	470
wai010	K	Ran	e	<***	8.83	0.1	400446.9	394499.9	0.1	<***	0.32	0.1		0.7	814.21	9.73	31/10	2635	470
wai012	K	Ran	c	21.36	42.44	33.01	400446.9	399102.7	4.23	24.95	5.74	4.75		0.76	1475.56	67.29	31/10	2443	460
wai012	K	Ran	e	50.11	44.94	23.39	400446.9	401109.9	0.1	0.44	3.6	7.04		0.82	1469.54	39.92	31/10	2443	460
wai013	K	Ran	c	****	25.69	0.48	400446.9	400298.9	0.96	3.18	33.92	6.47		0.34	1236.22	132.4	31/10	1833	420
wai013	K	Ran	e	11.72	8.49	0.1	400446.9	396990.3	0.25	<***	7.74	0.1		0.74	859.8	14.6	31/10	1833	420
wha006	K	Whm.	e	19.21	14.99	0.1	400447	409479.8	0.1	<***	****	0.1		0.48	1097.7	2.94	31/10	378	271
wha006	K	Whm.	c	<***	68.51	5.73	400447	403351.5	0.56	****	1.58	3.54		0.3	1148.64	2.78	31/10	378	271
29L	K	Aka	c	8.77	35.48	0.177	400804.3	391845.4	0.215	0.12	0.647	3.08	0.269	0.509	954.12	26.38	7/01	2233	430
29L	K	Aka	e	4.02	9.88	0.018	400804.3	390569.4	0.437	****	0.093	0.227	0.204	0.466	928.11	27.36	7/01	2233	430
32L	K	Aka	c	2.04	9.76	0.148	400804.3	395105.5	1.626	0.24	0.19	0.409	0.234	0.386	1496.28	57.81	7/01	3110	495
32L	K	Aka	e	5.7	6.25	0.061	400804.3	389802.3	0.036	0.04	0.033	0.32	0.272	0.477	834.6	10.18	7/01	3110	495
G023	K	Opu	c	6.71	42.33	0.177	400804.3	398913.8	1.221	0.8	5.49	1.72	0.167	0.244	1127.71	23.3	7/01	3346	535
G023	K	Opu	e	4.25	41.59	2.93	400804.3	407134.9	0.307	0.31	3.02	4.02	0.286	0.133	821.19	10.19	7/01	3346	535
G128	K	Opu	c	3.8	52.47	6.12	400804.2	387421.4	0.534	0.55	2.09	1.46	0.098	0.166	732.75	16.48	7/01	3549	490
G128	K	Opu	e	2.99	24.62	0.028	400804.3	395277.4	0.118	0.13	0.427	1.27	0.214	0.125	861.39	16.93	7/01	3549	490
10L	K	Ran	c	2.64	20.18	0.1	400804.3	387244.4	0.274	0.03	0.137	0.231	0.188	0.292	1423.61	57.11	7/01	1388	461
10L	K	Ran	e	6.12	9.75	0.1	400804.3	402034.7	0.189	0.06	0.034	0.046	0.267	0.303	1417.42	42.98	7/01	1388	461
11L	K	Ran	c	5.58	27.9	0.046	400804.3	388003.9	0.866	0.14	0.242	0.386	0.212	0.341	1439.19	77.05	7/01	2060	496

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
11L	K	Ran	e	5.66	9.4	1.2	400804.3	400028.9	0.262	0.07	0.033	0.049	0.247	0.609	1150.88	28.24	7/01	2060	496
2L	K	Ran	c	4.71	14.5	0.231	400804.3	401586.5	0.429	0.43	0.546	0.56	0.15	0.551	2470.87	30.91	7/01	3051	540
2L	K	Ran	e	5.06	8.86	0.1	400804.3	406075.3	0.316	0.42	0.218	0.1	0.19	0.668	1015.73	34.65	7/01	3051	540
4L	K	Ran	c	4.54	21.2	0.11	400804.3	400282.2	0.346	0.05	1.31	0.26	0.128	0.351	1634.86	83.03	7/01	1946	486
4L	K	Ran	e	15.68	9.72	0.25	400804.3	391911.8	0.207	0.26	0.165	1.7	0.079	0.779	1318.55	13.62	7/01	1946	486
5L	K	Ran	c	3.43	29.7	0.417	400804.3	391053.5	1.188	0.16	1.07	1.59	0.185	0.294	1505.31	55.48	7/01	1830	482
5L	K	Ran	e	5.59	11.6	0.096	400804.3	389981.5	0.131	0.08	0.202	0.69	0.233	0.345	1507.05	26.53	7/01	1830	482
7L	K	Ran	c	12.88	21.59	0.064	400804.3	396699.6	1.74	0.4	0.365	0.337	0.195	0.196	1442.07	80.84	7/01	1906	498
7L	K	Ran	e	8.93	6.88	0.032	400804.3	399780	0.059	0.11	0.103	0.07	0.217	0.286	1416.8	29.3	7/01	1906	498
9L	K	Ran	c	4.34	23.73	0.38	400804.3	393991.6	0.624	0.19	0.349	0.407	0.209	0.202	1482.94	68.16	7/01	1708	458
9L	K	Ran	e	15.19	13.51	0.01	400804.3	394909.7	0.074	0.11	0.136	0.35	0.121	0.322	1387.76	41.08	7/01	1708	458
34L	K	Waa	c	3.8	19.42	0.096	400804.3	393839.8	0.171	1.1	0.222	0.72	0.197	0.226	1544.45	23.58	7/01	2390	508
34L	K	Waa	e	12.39	13.71	1.37	400804.3	397798.5	0.193	0.54	0.96	4.59	0.154	0.146	1994.39	39.14	7/01	2390	508
36L	K	Waa	c	6.68	30.98	0.249	400804.3	389834.2	0.339	0.95	1.1	1.3	0.173	0.208	989.07	15.93	7/01	3119	569
36L	K	Waa	e	13	13.87	1.71	400804.3	397491	0.319	1.71	0.438	1.11	0.245	0.279	1644.86	16.11	7/01	3119	569
3L	K	Waa	c	3.84	30.83	0.037	400804.3	394835.4	1.333	0.11	0.216	0.333	0.184	0.33	1300.53	51.56	7/01	1469	405
3L	K	Waa	e	19.21	14.29	0.245	400804.3	395903.6	0.361	0.31	0.656	3.2	0.261	0.224	1940.73	24.52	7/01	1469	405
Waa006	K	Waa	c	2.05	28.95	0.1	400804.3	396369.3	0.237	0.58	0.557	0.35	0.188	0.154	1975.73	63.08	7/01		
Waa006	K	Waa	e	14.51	17.25	9.46	400804.3	392401.1	0.211	0.35	1.41	23.04	0.221	0.098	2146.79	28.91	7/01		
waa008	K	Waa	c	1.67	42.77	0.082	400804.3	416448.3	0.721	0.07	0.121	0.397	0.216	0.2	2010.94	28.57	7/01		

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
waa008	K	Waa	e	9.19	45.83	3.59	400804.3	395083.8	0.58	0.13	4.12	39.53	0.277	0.23	1896.54	45.57	7/01		
37L	K	Wai	c	3.89	18.42	0.006	400804.3	394344.3	0.303	0.42	0.653	0.08	0.179	0.231	1529.62	58.5	7/01	1320	430
37L	K	Wai	e	20.99	10.13	0.324	400804.3	392628.3	0.2	0.16	0.255	2.62	0.165	0.352	1373.16	38.71	7/01	1320	430
39L	K	Wai	c	12.26	28.93	1.17	400804.3	389355.7	1.024	1.67	4.89	8.14	0.239	0.233	1634.71	60.08	7/01	491	390
39L	K	Wai	e	11.49	9.75	0.496	400804.3	406068.7	0.09	0.74	1.43	6.61	0.233	0.336	1627.25	47.65	7/01	491	390
40L	K	Wai	c	4.28	23.56	0.068	400804.3	390400.2	4.24	0.01	0.113	0.281	0.1	0.258	1675.62	84.8	7/01	2377	514
40L	K	Wai	e	14.18	7.06	0.1	400804.3	387174.3	0.057	****	0.006	0.214	0.175	0.423	1105.61	15.01	7/01	2377	514
G114	K	Whm	c	15.76	32.11	0.72	400804.3	427144.8	0.303	****	0.95	3.09	0.192	0.18	1471.22	64.72	7/01	1100	370
G114	K	Whm	e	4	15.16	0.051	400804.3	417133.7	0.342	0.25	0.074	0.135	0.143	0.498	1320.66	50.87	7/01	1100	370
G202	K	Whm	c	6.06	53.79	0.143	400804.3	391463.3	0.41	0.18	0.639	0.96	0.2	0.294	1329.2	62.07	7/01	1450	398
G202	K	Whm	e	2.53	24.27	0.061	400804.3	411125.3	0.031	****	0.066	0.004	0.153	0.309	1087.59	52.84	7/01	1450	398
13L	K	Wha	c	9.9	20.35	0.705	400804.3	388093.9	0.544	0.35	0.602	0.85	0.208	0.37	1025.98	13.24	7/01	1834	459
13L	K	Wha	e	11.91	15.71	1.09	400804.3	407801.4	0.233	0.21	0.637	1.42	0.214	0.429	1290.22	19.46	7/01	1834	459
16L	K	Wha	c	2.82	15.76	0.16	400804.3	393124.7	0.198	0.39	1.12	0.194	0.242	0.42	1171.84	19.97	7/01	2050	455
16L	K	Wha	e	8.07	11.98	0.338	400804.3	393685.8	0.176	0.25	0.201	0.223	0.237	0.354	1391.19	16.91	7/01	2050	455
19L	K	Wha	c	5.03	19.99	0.1	400804.3	392866.7	1.293	0.24	1.18	0.121	0.162	0.192	1863.67	75.19	7/01	902	302
19L	K	Wha	e	9.57	9.74	0.1	400804.3	388255.7	0.091	****	0.143	1.35	0.251	0.202	1115.92	12.65	7/01	902	302
20L	K	Wha	c	1.11	23.21	0.062	400804.3	390777.2	2.06	0.31	0.058	0.263	0.246	0.221	1415.82	72.19	7/01	949	373
20L	K	Wha	e	2.34	9.94	0.1	400804.3	394835.1	0.089	0.14	0.809	0.061	0.158	0.197	1418.53	12.76	7/01	949	373
21L	K	Wha	c	1.54	16.26	0.057	400804.3	391150.8	0.123	0.59	0.075	0.127	0.254	0.225	1511.65	60.82	7/01	1200	403

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
21L	K	Wha	e	4.59	8.88	0.055	400804.3	387579.3	0.396	0.23	0.071	0.079	0.248	0.224	1364.42	67.75	7/01	1200	403
G065	K	Wha	c	2.19	34.19	0.391	400804.3	389310.4	0.5	0.34	0.421	0.46	0.13	0.155	1032.32	20.16	7/01	1197	380
G065	K	Wha	e	2.98	31.34	0.1	400804.2	391463.7	0.104	****	0.114	0.221	0.171	0.075	1663.63	18.04	7/01	1197	380
G138	K	Wha	c	4.99	25.66	0.46	400804.3	393717.8	0.289	0.67	0.5	0.77	0.206	0.175	910.49	12.15	7/01	1350	437
G138	K	Wha	e	2.92	20.63	0.563	400804.2	391733	0.322	0.21	62.35	13.66	0.181	0.076	1535.32	19.85	7/01	1350	437
wgf6	G	Mar	c	0.84	20.62	4.65	401006.9	400446.9	14.51	5.02	0.87	0.4	****	0.09	2069.44	123.46	19/03	7.08	69
wgf6	G	Mar	e	1.89	20.34	2.31	401266.4	400446.9	6.01	7.3	0.72	4.46	2.36	0.22	1988.72	132.6	19/03	7.08	69
wgf7	G	Mar	c	39.93	46.64	0.1	390097	400446.9	9.89	21.46	1.49	13.16	7.69	0.18	2007.32	91.03	19/03	11.58	82
wgf7	G	Mar	e	24	28.77	5.74	391976.4	400446.9	2.41	0.44	0.42	20.51	0.18	0.47	2008.3	103.19	19/03	11.58	82
fp11	G	Pun	c	<***	30.66	5.83	402144.3	400447	0.13	6.92	1.45	4.87	4.31	0.1	1432.79	54.48	19/03	13.85	85
fp11	G	Pun	e	45.29	37.73	12.03	402749.8	400447	1.19	****	0.07	10.03	<***	0.72	1506.04	83.66	19/03	13.85	85
wgf10	G	Whm	c	11.48	18.14	5.09	409066.2	400446.9	4.51	17.56	****	0.99	3.94	0.16	1665.41	58.13	19/03	6.11	67
wgf10	G	Whm	e	****	9.12	0.1	401855.6	400446.9	2.05	<***	2.03	4.12	<***	0.39	1690.51	64.29	19/03	6.11	67
wgf9	G	Whm	c	<***	13.8	0.1	396606	400446.9	7.86	4.4	****	3.2	<***	0.01	1589.41	56.33	19/03	5.89	68
wgf9	G	Whm	e	<***	66.69	10.18	397596.5	400446.9	17.92	0.7	1.3	9.15	1.18	0.37	1602.29	73.86	19/03	5.89	68
fp1	K	Pun	c	40.46	17.53	17.34	396445.2	400446.9	0.03	<***	3.44	0.1	<***	0.28	1351.87	45.48	19/03	0.75	33
fp1	K	Pun	e	16.66	89.05	8.76	401355	400446.9	1.18	****	0.74	19.92	1.05	0.48	1410.93	71.05	19/03	0.75	33
fp27	K	Pun	c	19.23	20.02	0.1	399675	400446.9	0.1	2.72	0.8	0.1	3.23	0.4	1304.99	65.83	19/03	1.26	40
fp27	K	Pun	e	44.76	36.27	0.1	399406.8	400446.9	0.7	11.04	0.45	6.56	1.18	0.1	1415.57	106.55	19/03	1.26	40
fp2	K	Pun	c	<***	39.04	0.1	402602.1	400446.9	0.4	<***	0.45	0.1	<***	0.1	1248.36	49.3	19/03	2.3	49

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
fp2	K	Pun	e	26.72	193.43	7.75	395080.2	400446.9	4.12	5.13	0.24	30.94	<***	0.21	1268.28	80.5	19/03	2.3	49
fp3	K	Pun	c	40.88	48.17	10.87	404086.8	400446.9	0.08	2.27	****	3.96	5.02	0.29	1218.11	44.57	19/03	2.21	47
fp3	K	Pun	e	<***	121.6	3.46	403329.2	400446.9	1.99	<***	0.23	27.76	0.16	0.27	1334.96	66.29	19/03	2.21	47
fp4	K	Pun	c	18.28	24.67	0.1	394539.4	400446.9	0.1	<***	0.72	3	2.1	0.43	1391.11	97.44	19/03	9.12	73
fp4	K	Pun	e	43.49	74.3	2.9	399047.6	400446.9	1.54	1.82	1.81	10.95	<***	0.19	1549.39	156.83	19/03	9.12	73
fp5	K	Pun	c	5.58	9.87	9.4	403116.5	400446.9	0.24	<***	1.45	0.1	<***	0.47	1497.78	54.15	19/03	8.46	67
fp5	K	Pun	e	13.63	60.81	0.1	397638	400446.9	2.46	<***	1.11	19.14	5.08	0.21	1565.53	154.38	19/03	8.46	67
fp6	K	Pun	c	58.12	22.21	20.44	407529	400446.9	0.1	17.63	****	3.39	4.1	0.1	1464.63	57.77	19/03	1.43	43
fp6	K	Pun	e	<***	19.23	2.41	396452.1	400446.9	0.51	<***	0.35	0.1	<***	0.25	1452.5	73.56	19/03	1.43	43
fe6a	K	Wai	c	13.5	18.52	4.48	405764.5	400446.9	0.1	9.1	1.04	4.11	0.39	0.57	1496.91	85.38	19/03	10	75
fe6a	K	Wai	e	4.29	115.2	9.9	397244.2	400446.9	0.81	4.97	4.68	20.36	0.34	0.34	1422.55	102.21	19/03	10	75
fp1a	K	Wai	c	<***	31.51	0.1	404711.3	400446.8	0.1	9.25	9.52	6.06	0.43	0.44	1461.27	44.45	19/03	3.6	56
fp1a	K	Wai	e	<***	21.48	9.27	407154.8	400446.8	0.04	11.13	****	0.1	<***	0.1	1492.79	56.77	19/03	3.6	56
fp2a	K	Wai	c	15.21	27.99	12.31	393106.9	400446.8	0.94	2.64	0.71	5.48	4.37	0.09	1417.06	42.36	19/03	6.6	66
fp2a	K	Wai	e	<***	47.9	8.89	397699.1	400446.8	0.3	<***	1.16	7.54	3.84	0.1	1411.17	62.83	19/03	6.6	66
fp3a	K	Wai	c	90.19	39.78	5.8	398228.4	400446.9	0.1	12.92	****	0.1	0.39	0	1310.69	44.25	19/03	5.2	67
fp3a	K	Wai	e	<***	17.82	0.1	396990.9	400446.9	0.38	<***	1.45	0.1	0.28	0.64	1263.28	43.88	19/03	5.2	67
fp5a	K	Wai	c	<***	18.34	0.1	399575.8	400446.9	0.51	****	****	2.03	2.39	0.09	1424.12	32.32	19/03	10	78
fp5a	K	Wai	e	<***	32.17	20.78	397653.3	400446.9	0.1	6.02	<***	13.77	****	0.33	1459.43	36.6	19/03	10	78
fp7a	K	Wai	c	44.27	974.48	10.99	405442.3	400446.9	0.54	0.3	1.4	3.74	4.59	0.12	373.16	5.3	19/03	2.24	47

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
fp7a	K	Wai	e	6.4	858.24	11.16	402533.8	400446.9	0.56	7.83	****	1.3	<***	0.1	365.63	7.14	19/03	2.24	47
g119	K	Wai	e	<***	40.77	4.76	408489.4	400446.9	1.22	0.29	0.18	0.1	4.46	0.57	1318.09	34.58	19/03	2073	490
g119	K	Wai	c	2.7	39.6	10.12	397565.7	400446.9	2.73	12.28	0.26	0.1	0.21	0.35	1247.96	65.45	19/03	2073	490
g120	K	Wai	c	33.3	59.8	7.74	394428.8	400446.9	0.1	<***	1.24	2.57	0.66	0.27	1202.29	45.32	19/03	1575	450
g120	K	Wai	e	6.27	30.94	0.1	413907.7	400446.9	0.59	3.42	****	2.89	****	0.31	1393	42.55	19/03	1575	450
g341	K	Wai	c	25.07	19.02	3.53	414901.5	400446.9	0.82	<***	1.24	5.3	0.25	0.27	1261.58	40.64	19/03	1983	445
g341	K	Wai	e	<***	5.6	0.1	413676.7	400446.9	0.1	<***	****	0.05	3.87	0.1	1305.21	38.58	19/03	1983	445
g40	K	Wai	c	<***	29.14	3.35	399780.6	400446.9	0.39	<***	1.22	4.64	1.25	0.33	1459.29	48.1	19/03	1293	397
g40	K	Wai	e	37.69	57.97	0.8	403116.9	400446.9	0.2	10.48	****	0.1	1.62	0.42	1625.54	30.9	19/03	1293	397
g132	K	Wha	c	<***	27.17	0.1	413987.3	400446.9	0.27	<***	0.59	3.45	<***	0.22	1149.91	13.14	19/03	2150	450
g132	K	Wha	e	<***	18.02	11.43	407322.5	400446.9	1.1	4.51	1.54	0.77	0.76	0.2	1074.68	39.09	19/03	2150	450
g242	K	Wha	c	2.89	19.77	3.86	407534.5	400446.9	0.24	<***	0.36	2.46	****	0.53	1178.98	34.57	19/03	1500	430
g242	K	Wha	e	<***	19.08	5.33	410317.8	400446.9	0.09	1.55	0.25	0.1	0.17	0.32	1061.3	20.69	19/03	1500	430
24L	K	Aka	c	15.19	21.83	2.07	400447	397455.7	0.36	0.55	0.68	0.1	****	0.63	894.05	14.91	2/04	1060	389
24L	K	Aka	e	10.03	12.07	0.23	400447	393373.6	0.05	2.32	0.62	0.8	0.91	0.58	957.64	18.7	2/04	1060	389
30L	K	Aka	c	23.5	14.1	0.1	400447	403255.6	0.86	6.49	0.9	0.31	0.59	0.03	939.54	31.01	2/04	2304	481
30L	K	Aka	e	6.63	13.45	0.1	400447	414682.2	0.34	10.62	****	1.29	2.09	0.39	830.84	12.69	2/04	2304	481
49R	K	Aka	c	20.02	46.95	1.77	400447	396247.4	0.79	<***	2.35	5.61	<***	0.42	1223.29	33.12	2/04	2537	443
49R	K	Aka	e	2.74	14.76	1.76	400447	406014	0.32	0.12	0.43	0.22	1.1	0.36	1294.69	17.95	2/04	2537	443
50L	K	Aka	c	44.71	32.22	0.1	400447	413371.7	2.02	2.97	0.48	0.58	****	0.17	1016.31	12.43	2/04	1549	438

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
50L	K	Aka	e	28.77	17.12	0.1	400447	400021.3	0.47	<***	****	3.12	0.98	0.37	1342.65	6.77	2/04	1549	438
51R	K	Aka	c	71.36	20.84	1.03	400447	401570.5	0.87	6.13	0.73	1.09	0.05	0.44	955.89	6.15	2/04	2313	439
51R	K	Aka	e	69.22	10.87	0.1	400447	400554.5	0.43	2.19	1.19	1.99	<***	0.18	1018.3	17.67	2/04	2313	439
52L	K	Aka	c	53.09	14.8	0.18	400447	400618.6	0.1	3.44	****	0.1	1.31	0.15	1425.51	17.37	2/04	2348	460
52L	K	Aka	e	70.95	10.19	0.1	400447	394502.8	0.3	1.91	****	0.87	0.99	0.66	1308.45	12.71	2/04	2348	460
53L	K	Aka	c	<***	35.23	0.1	400447	403595.8	0.9	1.66	1.09	1.05	1.45	1.07	1127.64	26.89	2/04	2497	459
53L	K	Aka	e	66.41	9.53	4.15	400447	398922.7	0.33	<***	****	2.37	****	0.99	964.06	20.52	2/04	2497	459
26L	K	Ran	c	<***	15.04	1.83	400447	404209.1	1.52	<***	0.28	1.89	****	0.49	1291.42	33.46	2/04	2880	509
26L	K	Ran	e	33.97	7.31	0.65	400447	399807.3	1.01	1.65	0.28	3.74	1.39	0.25	963.04	8.83	2/04	2880	509
25L	K	Waa	c	<***	18.66	0.1	400447	399680.4	0.07	0.64	0.63	0.1	2.21	0.7	1483.57	11.24	2/04	1622	432
25L	K	Waa	e	45.01	12.95	0.28	400447	396829.2	0.1	1.03	<***	2.25	3.48	0.66	2185.93	27.3	2/04	1622	432
27L	K	Waa	c	56.27	22.21	7.84	400447	400475.8	1.02	0.21	1.01	4.74	<***	0.59	2228.65	23.09	2/04	2417	486
27L	K	Waa	e	56.96	6.44	3.39	400447	403036.4	0.47	1.4	0.35	2.64	****	0.31	2425.79	26.86	2/04	2417	486
28L	K	Waa	c	15.89	15.91	0.1	400447	390567.9	0.1	****	0.63	0.64	5.22	0.16	1050.76	4.62	2/04	2458	527
28L	K	Waa	e	26.6	7.51	2.84	400447	405132.1	0.43	<***	0.23	1.15	0.17	0.6	1729.31	18.74	2/04	2458	527
33L	K	Waa	c	40.85	30.19	1.5	400447	399301.3	0.35	0.79	0.96	3.45	0.86	0.44	1096.75	21.02	2/04	1952	470
33L	K	Waa	e	<***	12.39	0.1	400447	404193.7	0.28	<***	****	1.74	<***	0.52	1010.35	15.42	2/04	1952	470
1L	K	Wai	c	21.78	19.44	1.69	400447	407291.1	0.04	<***	0.21	0.1	****	0.12	1337.81	28.36	2/04	1295	453
1L	K	Wai	e	10.87	13.57	26.02	400447	397320.7	0.02	<***	****	0.58	1.14	0.62	1543.09	37.55	2/04	1295	453
43L	K	Wai	c	<***	15.72	1.34	400447	390349.5	0.1	4.8	****	0.1	<***	0.94	1242.45	33.57	2/04	1808	469

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
43L	K	Wai	e	3.47	10.43	6.02	400447	398634.5	0.1	<***	2.88	4.67	<***	1.75	1282.98	26.27	2/04	1808	469
44L	K	Wai	c	0.38	13.71	0.09	400447	402106.9	0.08	<***	0.08	1.32	<***	0.2	1721.2	66.26	2/04	1323	452
44L	K	Wai	e	11.14	9.13	3.27	400447	414777.8	0.1	<***	0.46	1.68	0.7	0.1	1087.22	10.28	2/04	1323	452
45L	K	Wai	c	<***	20.85	1.13	400447	405131.3	1.77	1.81	0.55	2.59	1.01	0.33	1242.88	32.71	2/04	1110	409
45L	K	Wai	e	<***	10.72	1.45	400447	411501.9	0.28	0.5	0.12	0.38	1.73	0.31	965.16	5.93	2/04	1110	409
G123L	K	Wai	c	17.89	10.91	1.58	400447	403080.8	0.49	<***	0.38	4.34	<***	0.24	1292.46	46.36	2/04	1908	410
G123L	K	Wai	e	19.19	17.34	0.9	400447	395277.4	0.03	5.47	13.54	32.17	<***	0.32	1422.19	49.07	2/04	1908	410
54L	K	Whm	c	14.28	27.45	0.1	400447	406888.7	1.29	<***	0.52	0.76	1.34	0.34	1312.81	54.27	2/04	1425	386
54L	K	Whm	e	13.34	7.07	0.1	400447	397961.2	0.01	0	****	0.61	0.9	0.4	1143.86	11.29	2/04	1425	386
55L	K	Whm	c	<***	12.22	0.1	400447	396200.6	0.07	0.64	1.11	0.41	3.74	0.61	1319.97	41.5	2/04	1320	405
55L	K	Whm	e	7.4	5.6	3.64	400447	408313.8	0.22	0.16	0.8	0	1.29	0.19	1251.6	21.44	2/04	1320	405
56L	K	Whm	c	11.29	16.28	0.16	400447	403422.7	0.5	2.68	0.16	0.1	****	0.28	1372.06	22.52	2/04	1321	409
56L	K	Whm	e	<***	9.9	0.1	400447	401589.5	0.05	****	****	0.1	4.66	0.25	1234.3	21.34	2/04	1321	409
57L	K	Whm	c	14.95	8.52	1.71	400447	408202	0.1	<***	****	0.1	0.88	0.78	1251.02	57.78	2/04	1634	443
57L	K	Whm	e	54.97	12.96	3.2	400447	401326.8	0.1	<***	****	7.06	1.2	0.84	1486.72	56.43	2/04	1634	443
G362L	K	Whm	c	<***	117.41	2.97	400447	400382.5	2.34	1.84	1.6	3.86	2.09	0.33	900.86	19.21	2/04	1229	390
G362L	K	Whm	e	3.03	16.63	2.02	400447	403671.5	0.37	6.05	****	3.3	<***	0.02	1597.23	41.09	2/04	1229	390
G363L	K	Whm	c	25.24	12.85	0.05	400447	403895.9	0.71	<***	0.64	2.05	<***	0.47	1317.32	45.11	2/04	1090	344
G363L	K	Whm	e	73.88	17.19	0.63	400447	396441.1	0.4	<***	0.26	1.3	2	0.29	1259.11	45.35	2/04	1090	344
G243L	K	Wha	c	28.28	19.69	5.21	400447	400104.2	0.74	1.78	0.45	21.98	<***	0.5	1000.17	30.7	2/04	1700	440

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
G243L	K	Wha	e	35.24	52.4	4.1	400447	414434.7	0.27	10.19	44.98	70.84	0.81	0.24	1165.93	30.53	2/04	1700	440
wdf1L	G	Mar	c	<***	27.58	2.07	400447	394161.5	5.43	<***	0.37	0.79	5.41	0.63	1174.59	32.62	9/04	47.61	133
wdf1L	G	Mar	e	7.31	6.07	2.56	400447	403332.5	0.83	<***	0.82	5.42	3.54	0.46	1493.28	72.6	9/04	47.61	133
wgf5L	G	Mar	c	0.91	25.88	2.4	400447	403663.6	0.75	6.33	0.51	3.62	9.58	0.3	823.82	7.49	9/04	115.45	167
wgf5L	G	Mar	e	<***	6.75	3.82	400447	402039.3	0.7	****	0.87	0.1	1.86	0.49	1536.04	34.75	9/04	115.45	168
fp10	G	Pun	c	<***	38.31	1.2	400446.9	418325	1.58	13.51	0.89	1.79	2.28	0.13	1581.27	70.23	9/04	142	186
fp10	G	Pun	e	26.38	5.74	0.1	400446.9	411020.8	0.59	5.66	0.01	0.1	3.98	0.28	1255.99	88.25	9/04	142	186
fp11L	G	Pun	c	1.49	24.8	3.53	400446.9	414549.6	1.5	<***	0.66	5.31	1.88	0.67	1021.36	30.33	9/04	13.85	85
fp11L	G	Pun	e	55.98	21.54	1.09	400446.9	408971.4	1.28	****	<***	8.4	5.79	0.31	1323.33	121.76	9/04	13.85	85
fp18R	G	Pun	c	0.45	19.49	1.48	400446.9	411667.5	1.15	4.59	0.64	0.37	5.74	0.5	1377.89	47.01	9/04	199	211
fp18R	G	Pun	e	0.04	3.7	1.55	400446.9	409893.7	0.61	<***	****	5.25	****	0.17	1392.43	44.19	9/04	199	211
wdf2L	G	Whm	c	7.38	7.98	0.1	400447	402343.4	1.2	3.19	0.93	1.05	<***	0.31	1508.77	133.61	9/04	146.32	195
wdf2L	G	Whm	e	6.77	6.03	3.16	400447	405021.3	0.67	<***	0.22	21.82	<***	0.35	1067.4	30.63	9/04	146.32	196
wdf3L	G	Whm	c	<***	68.15	3.54	400447	402458.6	13.08	17.11	10.06	10.36	1.08	0.38	1491.4	102.44	9/04	77.7	151
wdf3L	G	Whm	e	7.73	6.25	1.9	400447	401750.9	2.47	5.9	0.88	0.1	2.28	0.61	1653.05	119.66	9/04	77.7	151
wgf4L	G	Whm	c	<***	21.97	1.5	400447	402125.6	2.93	<***	****	1.34	<***	0.26	1097.81	13.24	9/04	75.9	154
wgf4L	G	Whm	e	36.27	36.16	11.88	400447	407399	2.78	1.08	5.99	31.98	1.31	0.1	1079.78	115.48	9/04	75.9	154
wgf8L	G	Whm	c	<***	24.16	3.3	400447	404660	1.02	4.17	2.03	2.26	3.13	0.17	1447.2	52.78	9/04	29.57	111
Wgf8L	G	Whm	e	10.28	8.15	1.94	400447	405087.5	1.17	1.35	****	4.28	<***	0.59	1413.62	45.14	9/04	29.57	111
31L	K	Aka	c	23.4	24.48	0.76	400447	401756.3	1.02	4.02	****	1.43	2.75	0.44	1201.32	28.03	9/04	4843	591

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
31L	K	Aka	e	6.78	5.95	3.34	400447	406507.8	0.47	8.33	****	3.74	5.84	0.2	930.02	27.44	9/04	4843	591
48L	K	Aka	c	38.99	28.49	5.3	400447	402207.6	1.24	2.38	****	1.78	2.17	0.56	1044.69	28.89	9/04	1782	432
48L	K	Aka	e	45.32	13.07	1.72	400447	407820.3	0.09	****	1.31	0.1	<***	0.81	1015.65	15.5	9/04	1782	432
22L	K	Hak	c	14.87	16.73	1.21	400447	406124.1	1.13	5.36	0.76	5.11	2.73	0.18	1705.97	53.66	9/04	2399	475
22L	K	Hak	e	6.31	7.12	1.78	400447	412721.7	0.48	****	1.06	2.73	<***	0.21	2025.99	63.98	9/04	2399	475
wcarp1L	K	Mar	c	1.25	43.64	1.36	400447	403409	0.65	8.92	28.08	14.38	0.61	0.43	2305.7	31.04	9/04	47.61	133
wcarp1L	K	Mar	e	<***	7.13	1.6	400447	400015.7	0.95	2.67	0.45	0.1	4.85	0.31	1908.67	51.59	9/04	47.61	133
wcarp4L	K	Mar	c	0.72	13.06	0.73	400447	401703.3	0.21	1.96	1.18	4.37	****	0.06	1492.14	36.81	9/04	34	119
wcarp4L	K	Mar	e	15.54	9.14	3.38	400447	402489.7	0.1	<***	1.01	19.28	9.89	0.11	1734.66	44.81	9/04	34	119
fp12	K	Pun	c	27.73	42.97	7.43	400446.9	420377.9	0.1	1.12	1.04	5.88	****	0.14	1211.46	40.5	9/04	303	223
fp12L	K	Pun	e	<***	8.13	8.71	400446.9	411987.9	0.38	0.1	****	0.1	<***	0.59	1376.78	43.91	9/04	303	223
fp13L	K	Pun	c	33.47	68.73	3.74	400446.9	414056	0.76	<***	0.26	5.97	<***	0.42	1469.84	260.74	9/04	724	320
fp13L	K	Pun	e	68.67	13.56	2.53	400446.9	413915.5	0.37	5.08	0.23	28.57	4.24	0.32	1428.93	52.05	9/04	724	320
fp15L	K	Pun	c	4.42	26.21	2.77	400446.9	417427.6	0.17	****	0.38	0.1	2.56	0.49	1235.47	22.7	9/04	401	263
fp15L	K	Pun	e	23.63	11.94	2.79	400446.9	419431.1	0.19	1.71	0.74	5.12	****	0.41	1456.9	41.13	9/04	401	263
fp28L	K	Pun	c	<***	75.88	5.07	400446.9	422692	18.46	15.4	<***	0.1	5.16	0.7	1303.35	112.62	9/04	379	238
fp28L	K	Pun	e	8.66	8.48	1.99	400446.9	417106.7	0.32	<***	****	5.62	0.02	0.46	1446.91	68.64	9/04	379	238
fp29L	K	Pun	c	11.22	29.15	0.22	400446.9	413485.1	0.08	1.12	1.27	0.1	2.93	0.46	1412.86	607.91	9/04	748	318
fp29L	K	Pun	e	57.13	34.6	6.54	400446.9	415641.8	0.12	11	1.51	1.01	2.75	0.38	1019.03	32.86	9/04	748	318
fp30L	K	Pun	c	12.62	85.79	2.35	400446.9	415763.1	1.16	<***	0.03	3.39	3.4	0.47	958.34	39.4	9/04	262	226

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
fp30L	K	Pun	e	32.63	61.88	6.21	400446.9	412495.8	1.14	<***	****	9.08	<***	0.45	1312.66	55.09	9/04	262	226
fp31L	K	Pun	c	14.47	30.15	2.65	400446.9	417763.8	1	4.95	1.21	0.1	<***	0.86	1332.02	40.72	9/04	238	233
fp31L	K	Pun	e	29.44	29.45	1.07	400446.9	413613.8	1.18	<***	0.82	5.62	<***	1.28	1334.97	58.55	9/04	238	233
fp9L	K	Pun	c	41.3	64.07	0.1	400446.9	420644.1	3.32	<***	0.22	11.31	2.66	0.43	1195.93	47.55	9/04	286	225
fp9L	K	Pun	e	38.9	10.73	1.31	400446.9	415077.8	0.26	<***	****	0.88	2.85	0.7	1272.56	49.34	9/04	286	225
46L	K	Ran	c	21.26	22.91	0.56	400446.9	410489.2	0.49	6.91	1.39	3.94	3.71	0.46	1993.06	32.81	9/04	2303	482
46L	K	Ran	e	32.22	13.74	7.22	400446.9	411870.8	0.98	<***	1.12	0.79	<***	0.82	851.92	23.04	9/04	2303	482
6L	K	Ran	c	30.57	24.63	3.01	400446.9	415287.9	1.91	****	0.57	5.97	****	0.28	1492.7	35.54	9/04	2235	513
6L	K	Ran	e	0.22	10.99	4.28	400446.9	411454.8	0.02	5.72	****	0.1	0.59	0.17	1504.67	24.29	9/04	2235	513
33	K	Waa	c	24.9	14.12	1.4	400446.9	404847	0.48	2.84	****	0.1	3.28	0.18	1000.58	10.21	9/04	1952	470
33	K	Waa	e	<***	10.45	1.94	400446.9	406022	0.05	1.39	0.57	0.1	4.33	0.33	926.81	18.68	9/04	1952	470
35L	K	Waa	c	22.19	35.82	1.85	400447	406505.7	1.46	****	0.93	17.8	<***	0.01	980.56	9.41	9/04	3068	542
35L	K	Waa	e	27.7	8.42	2.26	400447	412748.1	0.1	6.54	0.13	1.01	1.46	0.29	1642.75	10.78	9/04	3068	542
38 L	K	Wai	c	12	10.85	2.59	400446.9	402130.6	0.28	<***	****	0.1	0.81	0.45	1236.36	36.85	9/04	1965	486
38 L	K	Wai	e	17.03	11.36	0.1	400446.9	394286.1	0.1	2.23	0.59	0.1	5.89	0.36	1228.53	23.18	9/04	1965	486
41L	K	Wai	c	26.29	16.55	1.37	400447	410196.5	0.1	0.95	0.8	0.08	7.26	0.28	1267.33	41.07	9/04	879	330
41L	K	Wai	e	21.24	9.66	4.05	400446.9	408383.4	0.96	5.83	****	0.1	2.22	0.21	1456.03	46.9	9/04	879	330
wcarp2L	K	Whm	c	1.98	63.98	1.8	400447	400925.9	1.73	12.88	14.79	13.37	<***	0.43	1140.31	32.69	9/04	52.3	137
wcarp2L	K	Whm	e	<***	14.74	0.52	400447	404490.8	1.39	0.81	0.71	0.1	<***	0.39	1296.66	54.09	9/04	52.3	137
wcarp3L	K	Whm	c	<***	78.87	2.92	400447	405867.1	1.83	<***	7.51	15.98	<***	0.21	1858.34	38.22	9/04	53.2	131

Appendix 10 (Continued) Raw untransformed LA-ICP-MS data, sorted by ablation date.

Name	Species	Capture site	Core/ edge	^{10}B	^{25}Mg	^{27}Al	^{42}Ca	^{43}Ca	^{55}Mn	^{62}Ni	^{65}Cu	^{66}Zn	^{75}As	^{85}Rb	^{88}Sr	^{137}Ba	Ablation date	Weight	Length
wcarp3L	K	Whm	e	<***	17.82	1.11	400447	397063.2	1.62	<***	0.29	2.59	<***	0.37	1298.11	49.66	9/04	53.2	131
14L	K	Wha	c	20.06	12.65	0.64	400446.9	413227.2	0.78	<***	****	0.1	5.22	0.33	1174.46	43.55	9/04	1748	456
14L	K	Wha	e	11.94	8.23	5.47	400446.9	403426.8	0.1	<***	0.76	4.06	5.88	0.11	1076.94	17.31	9/04	1748	456
15L	K	Wha	c	<***	14.87	4.22	400446.9	414278.7	0.1	<***	0.76	0.1	<***	0.1	1046.08	13.31	9/04	2666	510
15L	K	Wha	e	61.24	16.28	0.93	400446.9	415007	0.38	5.32	<***	1.63	1.67	0.49	653.56	7.65	9/04	2666	510
17L	K	Wha	c	18.35	29.73	2.67	400446.9	400483	1.06	<***	1.5	3.52	5.65	0.1	1193.81	21.11	9/04	1928	451
17L	K	Wha	e	2.68	11.99	3.44	400446.9	402148.3	0.47	****	<***	0.1	2.4	0.56	1074.21	26.67	9/04	1928	451
47L	K	Wha	c	17.75	21.31	0.05	400447	406716.3	2.09	<***	****	0.1	<***	0.18	884.97	18.78	9/04	1833	413
47L	K	Wha	e	30.64	14.17	4.31	400447	414406.9	0.68	<***	0.11	0.1	3.94	0.41	855.96	20.93	9/04	1833	413